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(54) Vav proto-oncogene protein.

(57) Nucleic acid sequences, particularly DNA sequences, coding for all or part of a *vav* proto-oncogene protein or for a modified *vav* proto-oncogene protein, expression vectors containing the DNA sequences, host cells containing the expression vectors, and methods utilizing these materials. The invention also concerns polypeptide molecules comprising all or part of a *vav* proto-oncogene protein or a modified *vav* proto-oncogene protein, and methods for producing these polypeptide molecules.

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Oncogenic activation has proven to be a valuable genetic marker for the identification of novel vertebrate genes [Varmus, H., Science 240, 1427-1435 (1988)]. The *ras* gene family, certain tyrosine protein kinases (*src* gene family, *abl, trk, met, ret*) and transcription factors (*fos, jun, erb*A) are just some of the best known examples. Although the precise function of these genes remains to be elucidated, their capacity to induce neoplasia strongly suggests that they play critical roles in the control of signal transduction processes [Bishop, J.M., Science 235, 305-311 (1987)].

The property of oncogenic activation has been used to isolate a number of novel human genes, one of which (*vav*) has been recently characterized at the molecular level. The *vav* gene was first identified when it became activated as an oncogene by a fortuitous rearrangement during the course of gene transfer assays [Katzav, S. et al., EMBO J. 8, 2283-2290 (1989)]. Molecular characterization of the human *vav* oncogene revealed a molecule capable of coding for a 797 amino acid polypeptide whose amino-terminus had been replaced by spurious sequences derived from the bacterial Tn5 gene used to confer G418 resistance to the transfected cells [Katzav, S. et al., supra]. The rest of the *vav* oncogene product contains a series of structural motifs reminiscent of those found in certain transcription factors, including a highly acidic amino-terminal region and a cystein-rich domain that depicts two putative metal binding structures [Johnson, P.F. et al., Annu. Rev. Biochem. 58, 799-839 (1989)].

The most intriguing feature of the *vav* gene is its pattern of expression. Analysis of *vav* gene transcripts in a series of human cell lines indicated that the *vav* gene is specifically expressed in cells of hematopoietic origin [Katzav, S. et al., supra]. No *vav* gene expression could be observed in either epithelial, mesenchymal or neuroectodermal cells. Interestingly, lymphoid, myeloid and erythroid cell lines contained comparable levels of *vav* gene transcripts. Similar results were obtained when normal human cells were examined, including B and T lymphocytes, macrophages and platelets [Katzav, S. et al., supra]. These observations suggest that the *vav* gene may play a basic role in hematopoiesis that is not influenced by differentiation programs.

It would be useful to isolate oncogenes from other mammalian species related to the human *vav* oncogene in order to more easily study the role of this protein in oncogenesis.

The present invention concerns an isolated nucleic acid molecule comprising a nucleic acid sequence coding for all or part of a mouse *vav* proto-oncogene protein. Preferably, the nucleic acid molecule is a DNA (deoxyribonucleic acid) molecule, and the nucleic acid sequence is a DNA sequence. Further preferred is a DNA sequence having all or part of the nucleotide sequence substantially as shown in Figure 2 [SEQ. ID NO: 1].

The present invention further concerns expression vectors comprising a DNA sequence coding for all or part of a mouse *vav* proto-oncogene protein.

The present invention additionally concerns prokaryotic or eukaryotic host cells containing an expression vector which comprises a DNA sequence coding for all or part of a mouse *vav* proto-oncogene protein.

The present invention also concerns methods for detecting nucleic acid sequences coding for all or part of a mouse *vav* proto-oncogene protein or related nucleic acid sequences.

The present invention further concerns polypeptide molecules comprising all or part of a mouse *vav* proto-oncogene protein.

Figure 1 shows a schematic diagram of a nucleotide sequence analysis of a mouse *vav* proto-oncogene cDNA clone. Untranslated 5' and 3' sequences are represented by a thin bar. Coding sequences are depicted by a thicker box and are flanked by the initiator (ATG) and terminator (TGA) codons. Highlighted domains include the leucine-rich domain (shaded box); the acidic region (black box) two proline-rich stretches (open box); two putative nuclear localization signals (left hatched box) and a cystein-rich region (right hatched box).

Figure 2 shows the nucleotide [SEQ. ID NO: 1] and deduced amino acid [SEQ. ID NO: 2] sequence of the 2793 bp insert of pMB24. The sequences of the flanking EcoRI linkers have been omitted. Numbers to the right of the sequence indicate nucleotide numbers and those to the left amino acid numbers. Underlined sequences correspond to those structures highlighted in (A). The cystein-rich domain has been boxed. Cysteine and histidine residues corresponding to the putative zinc finger-like structures (Cys-X_2-Cys-X_{13}-Cys-X_2-Cys and His-X_2-Cys-X_6-Cys-X_2-His) have been shaded. A putative protein kinase A phospho-rylation site is underlined by a crosshatched box and a putative polyadenylation signal by a wavy line.

Figure 3 shows detection of mouse *vav* gene transcripts. Two micrograms of poly A-selected RNA isolated from adult mouse tissue including (a) lung; (b) heart; (c) testes; (d) muscle; (e) intestine; (f) brain; (g) kidney; (h) spleen; (i) ovaries; (j) liver; and from murine cell lines including (k) NIH3T3 fibroblasts; (l) A20 B-lymphocyte and (m) MOPC 315 plasmacytoma cells were submitted to Northern transfer analysis. Nitrocellulose filters were hybridized under stringent conditions (50% v/v formamide, 42°C) to 5 x 10^7 cpm of a [^{32}P]-labeled nick-translated DNA fragment corresponding to the entire insert of pMB24. The hybridized filter was exposed to Kodak X-OMAT film for 24 hours at -70°C with the help of intensifier screens. *S. cerevisiae* 23S and 18S ribosomal RNAs were used as size markers. The migration of the 3 kb mouse *vav* proto-oncogene transcript is indicated

by a thick arrow.

Figure 4 shows identification of p95^{vav} as a mouse *vav* proto-oncogene product. [^{35}S methionine]-labeled cell extracts of (A) PAb 280, a mouse B-cell hybridoma; (B) PMM8, a mouse T-cell hybridoma; (C) NIH3T3 cells and (D) NIH3T3 cells transfected with pJC13, a pMEX-derived expression plasmid carrying a mouse *vav* proto-oncogene cDNA clone, were immunoprecipitated with (a) preimmune rabbit serum or (b) an antiserum raised against a peptide corresponding to a hydrophilic domain (amino acid residues 576-589) of a mouse *vav* protein either in the absence (-) or in the presence (+) of 10 μg of competing peptide. Immunoprecipitates were loaded onto 8% SDS-polyacrylamide gels. Electrophoresed gels were exposed to Kodak X-OMAT film for 2 days at -70°C in the presence of intensifier screens. The migration of p95^{vav} is indicated by a thick arrow. The migration of co-electrophoresed molecular weight standards including myosin (200,000), phosphorylase B (92,500) and bovine serum albumin (69,000) is also indicated.

Figure 5 shows the mechanism of activation of mouse and human *vav* oncogenes. Schematic representation of pMEX-derived expression vectors carrying normal and mutated *vav* cDNA clones. Symbols are those shown in Figure 1A. The presence of an MSV-LTR in each of these plasmids is also indicated. Bacterial Tn5-derived sequences present in the pSK27 plasmid containing a human *vav* oncogene [Katzav, S. et al., supra] are indicated by a dashed box. The [atg] symbol represent an in-frame translational initiator used by pJC12 and pJC7. This triplet codes for the methionine residues underlined in Figure 2. The right column indicates the relative transforming activity of these plasmids (expressed as focus forming units per microgram of linearized plasmid DNA) when tested in gene transfer assays using NIH3T3 cells as recipients.

Figure 6 shows that overexpression of wild type p95^{vav} protein can induce morphologic transformation of NIH3T3 cells. [^{35}S methionine]labeled cell extracts of (A) NIH3T3 cells; (B) NIH3T3 cells transformed by pJC13, an expression plasmid containing a full *vav* cDNA clone; (C) NIH3T3 cells transformed by pJC7, an expression plasmid containing a *vav* cDNA clone coding for a protein lacking the amino terminal domain (amino acid residues 1 to 65); and (D) NIH3T3 cells transformed by pSK27, an expression plasmid containing the human *vav* oncogene were immunoprecipitated with (a) normal rabbit serum and (b,c) a rabbit antiserum raised against a *vav* peptide either (b) in the presence or (c) in the absence of 10 μg of competing peptide. Immunoprecipitates were analyzed as indicated in the legend to Figure 4. The migration of the wild type p95^{vav} and the truncated p88^{vav} proteins is indicated by thick arrows. Co-electrophoresed molecular weight markers are those described in Figure 4 and ovalbumin (46,000).

Figure 7 shows the identification and mechanism of activation of a second human *vav* oncogene. DNAs (10 μg) isolated from (a) a nude mouse tumor induced by NIH3T3 cells that contain a human *vav* oncogene (Katzav, S. et al., supra); (b,c) nude mouse tumors induced by (b) second cycle- and (c) third cycle-transformants derived from transfection of NIH3T3 cells with human breast carcinoma DNA and (d) T24 human cells, were digested with Sac I and submitted to Southern transfer analysis. Hybridization was conducted for 48 hours under stringent conditions (50% v/v formamide, 42°C) using 5 x 10^7 cpm of [^{32}P]-labeled nick-translated probes corresponding to (A) a 180 bp EcoRI-Hinc II and (B) a 575 bp Sac I-Pst I DNA fragment of pSK65, a Bluescript-derived plasmid containing a human *vav* proto-oncogene cDNA clone [Katzav, S. et al., supra.]. Filters were exposed to Kodak X-OMAT film at -70°C for (A) 10 days or (B) 3 days in the presence of intensifier screens. Co-electrophoresed λ Hind III DNA fragments were used as size markers. The migration of the genomic (A) 4 kbp and (B) 7 kbp Sac I DNA fragments is indicated by arrows. The precise location of the pSK65-derived probes is indicated in the upper diagram. The vertical arrow indicates the breakpoint caused by the genomic rearrangement that activated the previously characterized human *vav* oncogene [Katzav, S. et al., supra].

The present invention concerns an isolated nucleic acid molecule comprising a nucleic acid sequence coding for all or part of a mouse *vav* proto-oncogene protein. Preferably, the nucleic acid molecule is a DNA molecule and the nucleic acid sequence is a DNA sequence. Further preferred is a DNA sequence having all or part of the nucleotide sequence substantially as shown in Figure 2 [SEQ. ID NO: 1], or a DNA sequence complementary to this DNA sequence. In the case of a nucleotide sequence (e.g., a DNA sequence) coding for part of a mouse *vav* proto-oncogene protein, it is preferred that the nucleotide sequence be at least about 15 nucleotides in length.

The DNA sequences of the present invention can be isolated from a variety of sources, although the presently preferred sequence has been isolated from two different mouse cDNA libraries. The exact amino acid sequence of the polypeptide molecule produced will vary with the initial DNA sequence.

The DNA sequences of the present invention can be obtained using various methods well-known to those of ordinary skill in the art. At least three alternative principal methods may be employed:

(1) the isolation of a double-stranded DNA sequence from genomic DNA or complementary DNA (cDNA) which contains the sequence;

(2) the chemical synthesis of the DNA sequence; and

(3) the synthesis of the DNA sequence by polymerase chain reaction (PCR).

In the first approach, a genomic or cDNA library can be screened in order to identify a DNA sequence coding for all or part of a mouse *vav* proto-oncogene protein. For example, a mouse cDNA library can be screened in order to identify a DNA sequence coding for all or part of a mouse *vav* proto-oncogene protein. Various mouse cDNA libraries, for example, those derived from WEHI-3 (ATCC TIB 68) cells and those derived from EL-4 (ATCC TIB 39) cells can be employed. Various techniques can be used to screen the genomic DNA or cDNA libraries.

For example, labeled single stranded DNA probe sequences duplicating a sequence present in the target genomic DNA or cDNA coding for all or part of a mouse *vav* proto-oncogene protein can be employed in DNA/DNA hybridization procedures carried out on cloned copies of the genomic DNA or cDNA which have been denatured to single stranded form.

A genomic DNA or cDNA library can also be screened for a genomic DNA or cDNA coding for all or part of a mouse *vav* proto-oncogene protein using immunoblotting techniques.

In one typical screening method suitable for either immunoblotting or hybridization techniques, the genomic DNA library, which is usually contained in a vector such as λGT11, or cDNA library is first spread out on agarose plates, and then the clones are transferred to filter membranes, for example, nitrocellulose membranes. A DNA probe can then be hybridized or an antibody can then be bound to the clones to identify those clones containing the genomic DNA or cDNA coding for all or part of a mouse *vav* proto-oncogene protein.

In the second approach, the DNA sequence of the present invention coding for all or part of a mouse *vav* proto-oncogene protein can be chemically synthesized. For example, the DNA sequence coding for a mouse *vav* proto-oncogene protein can be synthesized as a series of 100 base oligonucleotides that can then be sequentially ligated (via appropriate terminal restriction sites) so as to form the correct linear sequence of nucleotides.

In the third approach, the DNA sequences of the present invention coding for all or part of a mouse *vav* proto-oncogene protein can be synthesized using PCR. Briefly, pairs of synthetic DNA oligonucleotides at least 15 bases in length (PCR primers) that hybridize to opposite strands of the target DNA sequence are used to enzymatically amplify the intervening region of DNA on the target sequence. Repeated cycles of heat denaturation of the template, annealing of the primers and extension of the 3′-termini of the annealed primers with a DNA polymerase results in amplification of the segment defined by the 5′ ends of the PCR primers. See, U.S. Patent Nos. 4,683,195 and 4,683,202.

The DNA sequences of the present invention can be used in a variety of ways in accordance with the present invention. For example, they can be used as DNA probes to screen other cDNA and genomic DNA libraries so as to select by hybridization other DNA sequences that code for proteins related to a mouse *vav* proto-oncogene protein. In addition, the DNA sequences of the present invention coding for all or part of a mouse *vav* proto-oncogene protein can be used as DNA probes to screen other cDNA and genomic DNA libraries to select by hybridization DNA sequences that code for the *vav* proto-oncogene protein molecules from organisms other than mice.

The DNA sequences of the present invention coding for all or part of a mouse *vav* proto-oncogene protein can also be modified (i.e., mutated) to prepare various mutations. Such mutations may be either degenerate, i.e., the mutation does not change the amino acid sequence encoded by the mutated codon, or non-degenerate, i.e., the mutation changes the amino acid sequence encoded by the mutated codon. These modified DNA sequences may be prepared, for example, by mutating a mouse *vav* proto-oncogene protein DNA sequence so that the mutation results in the deletion, substitution, insertion, inversion or addition of one or more amino acids in the encoded polypeptide using various methods known in the art. For example, the methods of site-directed mutagenesis described in Taylor, J. W. et al., Nucl. Acids Res. 13, 8749-8764 (1985) and Kunkel, J. A., Proc. Natl. Acad. Sci. USA 82, 482-492 (1985) may be employed. In addition, kits for site-directed mutagenesis may be purchased from commercial vendors. For example, a kit for performing site-directed mutagenesis may be purchased from Amersham Corp. (Arlington Heights, IL). Both degenerate and non-degenerate mutations may be advantageous in producing or using the polypeptides of the present invention. For example, these mutations may permit higher levels of production, easier purification, or provide additional restriction endonuclease recognition sites. All such modified DNAs (and the encoded polypeptide molecules) are included within the scope of the present invention.

As used in the present application, the term "modified", when referring to a nucleotide or polypeptide sequence, means a nucleotide or polypeptide sequence which differs from the wild-type sequence found in nature.

The present invention further concerns expression vectors comprising a DNA sequence coding for all or part of a mouse *vav* proto-oncogene protein. The expression vectors preferably contain all or part of the DNA sequence having the nucleotide sequence substantially as shown in Figure 2 [SEQ. ID NO: 1]. Further preferred are expression vectors comprising one or more regulatory DNA sequences operatively linked to the DNA sequence coding for all or part of a mouse *vav* proto-oncogene protein. As used in this context, the term "operatively

linked" means that the regulatory DNA sequences are capable of directing the replication and/or the expression of the DNA sequence coding for all or part of a mouse *vav* proto-oncogene protein.

Expression vectors of utility in the present invention are often in the form of "plasmids", which refer to circular double stranded DNAs which, in their vector form, are not bound to the chromosome. However, the invention is intended to include such other forms of expression vectors which serve equivalent functions and which become known in the art subsequently hereto.

Expression vectors useful in the present invention typically contain an origin of replication, a promoter located in front of (i.e., upstream of) the DNA sequence and followed by the DNA sequence coding for all or part of a mouse *vav* proto-oncogene protein, transcription termination sequences and the remaining vector. The expression vectors may also include other DNA sequences known in the art, for example, stability leader sequences which provide for stability of the expression product, secretory leader sequences which provide for secretion of the expression product, sequences which allow expression of the structural gene to be modulated (e.g., by the presence or absence of nutrients or other inducers in the growth medium), marking sequences which are capable of providing phenotypic selection in transformed host cells, and sequences which provide sites for cleavage by restriction endonucleases. The characteristics of the actual expression vector used must be compatible with the host cell which is to be employed. For example, when cloning in a mmmalian cell system, the expression vector should contain promoters isolated from the genome of mammalian cells, (e.g., mouse metallothionien promoter), or from viruses that grow in these cells (e.g., vaccinia virus 7.5 K promoter). An expression vector as contemplated by the present invention is at least capable of directing the replication, and preferably the expression, of the DNA sequences of the present invention. Suitable origins of replication include, for example, the *Ori* origin of replication from the ColE1 derivative of pMB1. Suitable promoters include, for example, the long terminal repeats of the Moloney sarcoma virus, the Rous sarcoma virus and the mouse mammary tumor virus, as well as the early regions of Simian virus 40 and the polyoma virus. As selectable markers, the bacterial genes encoding resistance to the antibodies neomycin and G418 (*neo*) puromycin (*pur*) or hygromycin (*hygro*), or mammalian genes encoding thymidine kinase can be employed. All of these materials are known in the art and are commercially available.

Particularly preferred is the expression vector designated pMB24, described herein below, which contains the DNA sequence coding for a mouse *vav* proto-oncogene protein, or expression vectors with the identifying characteristics of pMB24.

E. coli host cells (strain XL1-Blue) containing the plasmid pMB24 were deposited with the American Type Culture Collection, Rockville, Maryland on January 23, 1991 under the Budapest Treaty and assigned ATCC accession no. 68516. pMB24 contains a cDNA clone of the mouse *vav* proto-oncogene encompassing the entire coding sequence.

Suitable expression vectors containing the desired coding and control sequences may be constructed using standard recombinant DNA techniques known in the art, many of which are described in Maniatis, T. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982).

The present invention additionally concerns host cells containing an expression vector which comprises a DNA sequence coding for all or part of a mouse *vav* proto-oncogene protein. The host cells preferably contain an expression vector which comprises all or part of the DNA sequence having the nucleotide sequence substantially as shown in Figure 2 [SEQ. ID NO: 1]. Further preferred are host cells containing an expression vector comprising one or more regulatory DNA sequences capable of directing the replication and/or the expression of and operatively linked to a DNA sequence coding for all or part of a mouse *vav* proto-oncogene protein. Suitable host cells include both prokaryotic and eukaryotic cells. Suitable prokaryotic host cells include, for example, various strains of *E. coli* such as DH5, C600 and LL1. Suitable eukaryotic host cells include, for example, mouse NIH3T3 and BALB3T3 cells, rat Rat-2 cells, monkey COS cells, human Hela cells and hamster CHO cells.

Preferred as host cells are mouse NIH3T3 cells.

Expression vectors may be introduced into host cells by various methods known in the art. For example, transfection of host cells with expression vectors can be carried out by the calcium phosphate precipitation method. However, other methods for introducing expression vectors into host cells, for example, electroporation, biolistic fusion, liposomal fusion, nuclear injection and viral or phage infection can also be employed.

Once an expression vector has been introduced into an appropriate host cell, the host cell can be cultured under conditions permitting expression of large amounts of the desired polypeptide, in this case a polypeptide molecule comprising all or part of a mouse *vav* proto-oncogene protein. Such polypeptides are useful in the study of the characteristics of a mouse *vav* proto-oncogene protein, for example, its role in oncogenesis. Such polypeptides can also be used to identify potential anti-cancer drugs. For example, a compound which is able to bind to or inhibit the function of the *vav* proto-oncogene may be an effective cancer chemotherapeutic agent.

Host cells containing an expression vector which contains a DNA sequence coding for all or part of a mouse

vav proto-oncogene protein may be identified by one or more of the following four general approaches: (a) DNA-DNA hybridizaiton; (b) the presence or absence of marker gene functions; (c) assessing the level of transcription as measured by the production of mouse *vav* proto-oncogene protein mRNA transcripts in the host cell; and (d) detection of the gene product immunologically.

In the first approach, the presence of a DNA sequence coding for all or part of a mouse *vav* proto-oncogene protein can be detected by DNA-DNA or RNA-DNA hybridization using probes complementary to the DNA sequence.

In the second approach, the recombinant expression vector host system can be identified and selected based upon the presence or absence of certain marker gene function (e.g., thymidine kinase activity, resistance to antibiotics, etc.). A marker gene can be placed in the same plasmid as the DNA sequence coding for all or part of a mouse *vav* proto-oncogene protein under the regulation of the same or a different promoter used to regulate a mouse *vav* proto-oncogene protein coding sequence. Expression of the marker gene in response to induction or selection indicates expression of the DNA sequence coding for all or part of a mouse *vav* proto-oncogene protein

In the third approach, the production of mouse *vav* proto-oncogene protein mRNA transcripts can be assessed by hybridization assays. For example, polyadenylated RNA can be isolated and analyzed by Northern blotting or nuclease protection assay using a probe complementary to the RNA sequence. Alternatively, the total nucleic acids of the host cell may be extracted and assayed for hybridization to such probes.

In the fourth approach, the expression of all or part of a mouse *vav* proto-oncogene protein can be assessed immunologically, for example, by Western blotting.

The DNA sequences of expression vectors, plasmids or DNA molecules of the present invention may be determined by various methods known in the art. For example, the dideoxy chain termination method as described in Sanger et al., Proc. Natl. Acad. Sci. USA 74, 5463-5467 (1977), or the Maxam-Gilbert method as described in Proc. Natl. Acad. Sci. USA 74, 560-564 (1977) may be employed.

It should, of course, be understood that not all expression vectors and DNA regulatory sequences will function equally well to express the DNA sequences of the present invention. Neither will all host cells function equally well with the same expression system. However, one of ordinary skill in the art may make a selection among expression vectors, DNA regulatory sequences, and host cells using the guidance provided herein without undue experimentation and without departing from the scope of the present invention.

The present invention further concerns a method for detecting a nucleic acid sequence coding for all or part of a mouse *vav* proto-oncogene protein or a related nucleic acid sequence comprising contacting the nucleic acid sequence with a detectable marker which binds specifically to at least a portion of the nucleic acid sequence, and detecting the marker so bound. The presence of bound marker indicates the presence of the nucleic acid sequence. Preferably, the nucleic acid sequence is a DNA sequence having all or part of the nucleotide sequence substantially as shown in Figure 2 [SEQ. ID NO: 1]. Also preferred is a method in which the DNA sequence is a genomic DNA sequence. A DNA sample containing the DNA sequence may be isolated using various methods for DNA isolation which are well-known to those of ordinary skill in the art. For example, a genomic DNA sample may be isolated from tissue by rapidly freezing the tissue from which the DNA is to be isolated, crushing the tissue to produce readily digestible pieces, placing the crushed tissue in a solution of proteinase K and sodium dodecyl sulfate, and incubating the resulting solution until most of the cellular protein is degraded. The digest is then deprotenized by successive phenol/chloroform/isoamyl alcohol extractions, recovered by ethanol precipitation, and dried and resuspended in buffer.

Also preferred is the method in which the nucleic acid sequence is an RNA sequence. Preferably, the RNA sequence is an mRNA sequence. Additionally preferred is the method in which the RNA sequence is located in the cells of a tissue sample. An RNA sample containing the RNA sequence may be isolated using various methods for RNA isolation which are well-known to those of ordinary skill in the art. For example, an RNA sample may be isolated from cultured cells by washing the cells free of media and then lysing the cells by placing them in a 4 M guanidinium solution. The viscosity of the resulting solution is reduced by drawing the lysate through a 20 gauge needle. The RNA is then pelleted through a $CsCl_2$ step gradient, and the supernatant fluid from the gradient carefully removed to allow complete separation of the RNA, found in the pellet, from contaminating DNA and protein.

The detectable marker useful for detecting a nucleic acid sequence coding for all or part of a mouse *vav* proto-oncogene protein or a related nucleic acid sequence, may be a labeled DNA sequence, including a labeled cDNA sequence, having a nucleotide sequence complementary to at least a portion of the DNA sequence coding for all or part of a mouse *vav* proto-oncogene protein.

The detectable marker may also be a labeled sense or antisense RNA sequence having a nucleotide sequence complementary to at least a portion of the DNA sequence coding for all or part of a mouse *vav* proto-oncogene protein

The detectable markers of the present invention may be labeled with commonly employed radioactive labels, such as ^{32}P and ^{35}S, although other labels such as biotin or mercury may be employed. Various methods well-known to those of ordinary skill in the art may be used to label the detectable markers. For example, DNA sequences and RNA sequences may be labeled with ^{32}P or ^{35}S using the random primer method.

Once a suitable detectable marker has been obtained, various methods well-known to those of ordinary skill in the art may be employed for contacting the detectable marker with the sample of interest. For example, DNA-DNA, RNA-RNA and DNA-RNA hybridizations may be performed using standard procedures known in the art. In a typical DNA-DNA hybridization procedure for detecting DNA sequences coding for all or part of a mouse *vav* proto-oncogene protein in genomic DNA, the genomic DNA is first isolated using known methods, and then digested with one or more restriction enzymes. The resulting DNA fragments are separated on agarose gels and denatured *in situ*. After prehybridization to reduce nonspecific hybridization, a radiolabeled nucleic acid probe is hybridized to the immobilized DNA fragments. The filter is then washed to remove unbound or weakly bound probe, and is then auto-radiographed to identify the DNA fragments that have hybridized with the probe.

The presence of bound detectable marker may be detected using various methods well-known to those of ordinary skill in the art. For example, if the detectable marker is radioactively labeled, autoradiography may be employed. Depending on the label employed, other detection methods such as spectrophotometry may also be used.

It should be understood that nucleic acid sequences related to nucleic acid sequences coding for all or part of squalene synthetase can also be detected using the methods described herein. For example, a DNA probe based on conserved regions of a mouse *vav* proto-oncogene protein (e.g., the helix-loop region, leucine zipper domain and cystein-rich [zinc-finger] domain) can be used to detect and isolate related DNA sequences (e.g., a DNA sequence coding for a rat *vav* proto-oncogene protein). All such methods are included within the scope of the present invention.

As used in the present application and in this context, the term "related" means a nucleic acid sequence which is able to hybridize to an oligonucleotide probe based on the nucleotide sequence of a mouse *vav* proto-oncogene protein.

The present invention further concerns polypeptide molecules comprising all or part of a mouse *vav* proto-oncogene protein, said polypeptide molecules preferably having all or part of the amino acid sequence substantially as shown in Figure 2 [SEQ. ID NO: 2].

The polypeptides of the present invention may be obtained by synthetic means, i.e., chemical synthesis of the polypeptide from its component amino acids, by methods known to those of ordinary skill in the art. For example, the solid phase procedure described by Houghton et al., Proc. Natl. Acad. Sci. 82, 5135 (1985) may be employed. It is preferred that the polypeptides be obtained by production in prokaryotic or eukaryotic host cells expressing a DNA sequence coding for all or part of a mouse *vav* proto-oncogene protein, or by *in vitro* translation of the mRNA encoded by a DNA sequence coding for all or part of a mouse *vav* proto-oncogene protein. For example, the DNA sequence of Figure 2 [SEQ. ID NO: 1] may be synthesized using PCR as described above and inserted into a suitable expression vector, which in turn may be used to transform a suitable host cell. The recombinant host cell may then be cultured to produce a mouse *vav* proto-oncogene protein. Techniques for the production of polypeptides by these means are known in the art, and are described herein.

The polypeptides produced in this manner may then be isolated and purified to some degree using various protein purification techniques. For example, chromatographic procedures such as ion exchange chromatography, gel filtration chromatography and immunoaffinity chromatography may be employed.

The polypeptides of the present invention may be used in a wide variety of ways. For example, the polypeptides may be used to prepare in a known manner polyclonal or monoclonal antibodies capable of binding the polypeptides. These antibodies may in turn be used for the detection of the polypeptides of the present invention in a sample, for example, a cell sample, using immunoassay techniques, for example, radioimmunoassay or enzyme immunoassay. The antibodies may also be used in affinity chromatography for purifying the polypeptides of the present invention and isolating them from various sources.

The polypeptides of the present invention have been defined by means of determined DNA and deduced amino acid sequencing. Due to the degeneracy of the genetic code, other DNA sequences which encode the same amino acid sequence as depicted in Figure 2 [SEQ. ID NO: 2] may be used for the production of the polypeptides of the present invention. In addition, it will be understood that allelic variations of these DNA and amino acid sequences naturally exist, or may be intentionally introduced using methods known in the art. These variations may be demonstrated by one or more amino acid differences in the overall sequence, or by deletions, substitutions, insertions, inversions or additions of one or more amino acids in said sequence. Such amino acid substitutions may be made, for example, on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphiphathic nature of the residues involved. For example, negatively charged amino

acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; amino acids with uncharged polar head groups or nonpolar head groups having similar hydrophilicity values include the following: leucine, isoleucine, valine; glycine, alanine; asparagine, glutamine; serine, threonine; phenylalanine, tyrosine. Other contemplated variations include salts and esters of the aforementioned polypeptides, as well as precursors of the aforementioned polypeptides, for example, precursors having N-terminal substituents such as methionine, N-formylmethionine and leader sequences. All such variations are included within the scope of the present invention.

The following examples are further illustrative of the present invention. These examples are not intended to limit the scope of the present invention, and provide further understanding of the invention.

EXAMPLE I

ISOLATION AND CHARACTERIZATION OF MOUSE *VAV* PROTO-ONCOGENE

A. MATERIALS AND METHODS

1. Gene Transfer Assay

NIH3T3 mouse cells were transfected with various amount (1 ng to 1 µg) of linearized plasmid DNA in the presence of 20 µg of carrier (calf thymus) DNA as described in Graham, F.L. and van der Eb, A.J., Virology 52, 456-467 (1975). Foci of transformed cells were scored after 14 days. To isolate G418-resistant colonies, NIH3T3 cells were co-transfected with 20 ng of pSV*neo* DNA and 1 µg of the desired plasmid DNA as described in Fasano, O. et al., Mol. Cell Biol. 4, 1695-1705 (1984).

2. Mouse *vav* cDNA clones

cDNA libraries derived from WEHI-3 and EL-4 hematopoietic cell lines (Stratagene, La Jolla, CA) were screened under partially relaxed hybridization conditions (42°C in 5 X SSC [SSC = 35.06 g/l NaCl, 17.65 g/l Na-citrate, pH 7.0], 40% formamide, 1 X Denhardt's solution) using as a probe a [^{32}P]-labeled insert of pSK8 (ATCC 41060), a plasmid containing a partial cDNA clone of the human *vav* proto-oncogene [Katzav, S. et al., supra]. Recombinant phages carrying the longest inserts (2.8 kbp) were subcloned [GIVE SOME DETAILS] in Bluescript KS (Stratagene) to generate pMB24 and pMB25. These mouse *vav* cDNA clones were submitted to nucleotide sequence analysis by the dideoxy chain termination method [Sanger, F. et al., Proc. Natl. Acad. Sci. USA 74, 5463-5467 (1977)] using double-stranded DNA, synthetic oligonucleotides as primers and modified T7 DNA polymerase (Sequenase, United States Biochemicals, Cleveland, OH).

3. Expression plasmids

Mouse *vav* expression plasmids. pJC11 was generated by subcloning the entire 2.8 kbp cDNA insert of pMB24 into the EcoRI site of pMEX, a mammalian expression vector carrying a multiple cloning site flanked by an MSV LTR (Maloney sarcoma virus, long terminal repeat) and a SV40 polyadenylation signal [Martin-Zanca, D. et al., Mol. Cell Biol. 9, 24-33 (1989)]. Subcloning procedures involved digestion of pMB24 DNA with the restriction endonuclease Eco RI, purification of the 2.8 kbp cDNA insert and religation to Eco RI-digested pMEX DNA. These procedures are standard recombinant DNA techniques and are described in detail in Maniatis, T. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982). The 2.8 kbp EcoRI DNA insert of pMB24 was isolated after partial digestion to avoid cleavage at the internal EcoRI site (nucleotides 2251-2256, Figure 2) [see SEQ. ID NO: 1]. pJC12 was obtained by deleting an internal 280 bp DNA fragment encompassed between the Sal I cleavage site present in the MCS and the unique Nru I site located at position 184-189 (Figure 2) [see SEQ. ID NO: 1]. This Nru I site lies just upstream of a second ATG codon (nucleotides 209-211, Figure 2) [see SEQ. ID NO: 1] that serves as a translational initiator in this plasmid. pJC17 was generated by replacing the internal 607 bp Kpn I-Stu I DNA fragment (nucleotides 992-1599 in Figure 2) [see SEQ. ID NO: 1] of pJC12 by a mutant DNA fragment carrying a single point mutation (T→A) at position 1595 (Figure 2) [SEQ. ID NO: 1]. The mutated fragment was obtained by PCR-aided amplification of the 607 bp Kpn I-Sru I DNA fragment using a mismatched 3' amplimer. pJC18 was generated by replacing the internal 186 bp Eco RV-Bam HI DNA fragment (nucleotide 1638-1824 in Figure 2) [see SEQ. ID NO: 1] of pJC12 with a mutant DNA fragment carrying a single point mutation (G→C) at position 1738 (Figure 2) [see SEQ. ID NO: 1]. The mutated fragment was obtained by PCR-aided amplification of the 186 bp Eco RV-Bam HI DNA fragment using a mismatched 5' amplimer. pJC19 was generated by replacing the internal

72 bp Eco RV-Nco I DNA fragment (nucleotides 1638-1800 in Figure 2) [see SEQ. ID NO: 1] of pJC12 by a mutant DNA fragment carrying a single point mutation (C→G) at position 1670 (Figure 2) [see SEQ. ID NO: 1]. The mutated DNA fragment was obtained by chemical synthesis.

Human *vav* gene expression plasmids. pJC7 was obtained by inserting the 2.9 kbp EcoRI cDNA clone of pSK65 [Katzav, S. et al., supra] into the unique EcoRI site of pMEX. pJC13 was obtained by replacing the internal 850 bp Pst I DNA fragment of pJC7 by a similar DNA fragment generated by PCR-aided amplification using a 5′ amplimer

(5'CCGGCTGCAGGCCACCATGGAGCTGTGGCGCCAATGCACC3')

that carried an insertion of four nucleotides (underlined). The inserted bases reconstitute the coding sequences presumably missing in pJC7. pJC15 was obtained by replacing the internal 552 bp Bal I fragment of pJC7 by a similar PCR-generated DNA fragment carrying a single point mutation (T→C) in the triplet coding for the first cysteine residue of the first zinc-finger like structure (Table 2). To obtained the mutated 552 bp Bal I fragment., an 87 bp Bal I-Stu I fragment was amplified by PCR using a 3′ amplimer that carried the mismatch needed to introduce the required T→C mutation. This PCR-generated BalI-StuI fragment was then ligated to the wild type 465 bp Stu I-Bal I DNA fragment obtained from pJC7. The nucleotide sequence of each of the above expression plasmids was verified by direct sequencing of double stranded DNA. Moreover, these expression plasmids directed the synthesis of the expected *vav* protein as determined by immunoprecipitation analysis of G418-resistant NIH3T3 cells generated by co-transfection of these plasmids with the selectable marker pSV2neo.

4. Southern and Northern blot analysis

High molecular weight DNA was digested to completion with appropriate restriction endonucleases, electrophoresed in 0.7% agarose gels and submitted to Southern transfer analysis as described in Southern, E.M., J. Mol. Biol. 98, 503-517 (1975). Total cellular RNA was extracted by the guanidium thiocyanate method [Chirgwin, J.M. et al., Biochemistry 18, 5294-5299 (1979)] and purified by centrifugation through cesium chloride. Poly(A)-containing RNA was isolated by retention on oligo(dT) columns (Collaborative Research, Bedford, MA). Total RNA (10 μg) or poly(A)-selected RNA (3 μg) were submitted to Northern transfer analysis as described in Lehrach, H. et al., Biochemistry 16, 4743-4751 (1977). The nitrocellulose filters were hybridized with various ^{32}P-labeled nick translated probes for 48 hours under stringent conditions (42°C in 5 X SSC, 50% formamide, 1 X Denhardt's solution).

5. Protein analysis

Transfection of NIH3T3 cells, isolation of transformed cells, selection of G418-resistant colonies, metabolic labeling of cells with [^{35}S-]-methionine, immunoprecipitation with various antisera and SDS-PAGE analysis were carried out as described in Martin-Zanca, D. et al., Mol. Cell Biol. 9, 24-33 (1989). The rabbit antiserum used to immunoprecipitate the *vav* proteins was raised against a synthetic 14-mer peptide (KDKLHRRAQDKKRN) whose sequence corresponds to either amino acid residues 576 to 589 of a mouse *vav* protein (Figure 1) or to residues 528 to 541 of the human *vav* oncogene product [Katzav, S. et al., supra].

B. RESULTS

1. Nucleotide sequence of the mouse *vav* proto-oncogene

Independent mouse cDNA libraries derived from two hematopoietic cell lines (WEHI-3 and EL-4) were used to isolate cDNA clones of the mouse *vav* proto-oncogene. WEHI-3 (ATTC TIB 68) is a myeloid cell line and EL-4 (ATCC TIB 39) cells were established from a mouse T-cell lymphoma. A total of 12 cDNA clones were isolated. Those recombinant phages containing the longest inserts from each library (2792 Kbp from the WEHI-3 and 2788 Kbp from the EL-4 cDNA library) were excised by using a helper phage, circularized and propagated in *E. coli* DH5 cells as plasmids. These plasmids, designated pMB24 (WEHI-3 library) and pMB25 (EL-4 library) were subsequently submitted to nucleotide sequence analysis using standard dideoxy sequencing techniques as described in Sanger et al., supra.

Figure 2 [SEQ. ID NO: 1] depicts the nucleotide sequence of the 2,793 bp long insert of pMB24. pMB25, the cDNA clone derived from EL-4 T-cell cDNA library possessed an identical sequence extending from nucleotide 5 to 2792. These results indicate that these cDNA clones are faithful representatives of normal *vav* transcripts in mouse hematopoietic cells. Analysis of the nucleotide sequence of pMB24 revealed a long open

reading frame extending from nucleotides 14 to 2597. The first in-frame ATG codon (nucleotides 14-16) is part of the canonical GCCACCATGG motif characteristic of efficient mammalian translational initiators [Kozak, M., Nucleic Acids Res. 15, 8125-8148, (1987)]. Analysis of mouse *vav* cDNA clones carrying additional 5' sequences revealed an inframe terminator codon (TGA) 45 nucleotides upstream of the beginning of the pMB24 clone (Figure 2) [see SEQ. ID NO: 1]. Therefore, it is likely that *vav* protein synthesis initiates at this ATG codon. If so, a mouse *vav* proto-oncogene would code for an 844 amino acid-long polypeptide with a predicted molecular mass of 97,303 daltons. This open reading frame is followed by a stretch of 195 bp of 3' non-coding sequences which includes a translational terminator TGA (nucleotides 2598-2600) and the concensus polyadenylation signal AATAAA (positions 2774 to 2779) (Figure 2) [see SEQ. ID NO: 1]. Analysis of additional mouse *vav* cDNA clones carrying additional 3' sequences revealed the presence of a polyA tail just two nucleotides downstream from the end of clone pMB24.

The predicted amino acid sequence of the putative 844 amino acid-long mouse *vav* protein revealed a leucine-rich domain extending from amino acid residues 33 to 102 (Figure 2) [see SEQ. ID NO: 2] . This domain includes a short sequence, Ala-Leu-Arg-Asp-X-Val which is also present in each of the three members of the *myc* oncogene family. This conserved motif is located within an amphipathic helix-loop-helix domain, which in *myc* proteins is required for dimerization and DNA binding [Murre, C. et al., Cell 56, 777-783 (1989)]. This sequence, however, is not shared by other DNA binding proteins such as *Myo* D1, *daughterless* and one of the members of the *achaete-scute* complex that exhibit similar helix-loop-helix motifs [Murre, C. et al., Cell 58, 537-544 (1989)]. The amino terminal leucinerich domain of the *vav* proto-oncogene has additional structural homologies with the members of the *myc* gene family. They include a heptad repeat of hydrophobic residues, of which three (four in the *myc* proteins) are leucines. This leucine zipperlike domain is separated from the shared Ala-Leu-Arg-Asp-X-Val sequence by a putative hinge region that contains two proline residues. A similar combination of helix-loop-helix structure followed by a heptad repeat of hydrophobic sequences has been shown to be involved in ligand binding and dimerization of nuclear receptors [Fawell, S.E. et al., Cell 60, 953-962 (1990)].

Other relevant features identified in the deduced amino acid sequence of a mouse *vav* proto-oncogene product include: (i) a highly acidic 45 amino acid-long domain (residues 132-176) in which 22 residues (49%) are either glutamine or aspartic acid; (ii) two stretches of proline residues (positions 336 to 340 and 606 to 609) that may represent hinge regions; (iii) a putative protein kinase A phosphorylation site (residues 435 to 440); (iv) two putative nuclear localization signals (residues 486 to 493 and 575 to 582); (v) a cysteine-rich sequence which includes two metal binding motifs Cys-X_2-Cys-X_{13}-Cys-X_2-Cys (residues 528 to 548) and His-X_2-Cys-X_6-Cys-X_2-His (residues 553 to 566). The former is similar to zinc finger motif found in transcriptional activators such as the adenovirus E1A, yeast GAL4 and certain steroid receptors [Johnson et al., Annu. Rev. Biochem. 58 799-839 (1989)]. The overall alignment of cysteine residues in this domain (Cys-X_2-Cys-X_{13}-Cys-X_2-Cys-X_7-Cys-X_6-Cys) is also reminiscent of the tandem motifs found in the amino terminal domain of the various members of the protein kinase C family and in a diacyglycerol kinase [Coussens et al., Science 233 859-866 (1986) and Sakane, F. et al., Nature 344 345-348 (1990)].

2. Homology with the human *vav* oncogene

Alignment of the deduced amino acid sequences of a mouse and human *vav* gene products reveal a remarkable degree of homology. The predicted mouse *vav* proto-oncogene sequence (amino acid residues 3 to 844) is 91.2% identical (769 residues) to that of its human counterpart. Of the 73 different residues, at least 30 are conservative substitutions, thus yieldig an overall homology of 94.8% between human and murine *vav* proteins. More importantly, all of the other relevant domains previously identified in the product of the human *vav* gene, including the acidic domain, the two proline hinge regions, the putative protein kinase A phosphorylation site, the cystein-rich sequence that can fold into zinc finger-like structures and the putative nuclear localization signals, are also present in a mouse *vav* gene product (Figure 2) [see SEQ. ID NO: 1] . The mouse *vav* protein is one amino acid shorter (844 residues) due to the presence of a single Ile^{717} residue instead of the sequence Thr^{717} Val^{718} found in its human counterpart.

Comparison of a mouse *vav* proto-oncogene product with that of the human *vav* oncogene suggest that its 67 amino terminal amino acids were replaced by 19 unrelated residues derived from the bacterial Tn5 gene. Therefore, the human *vav* oncogene retains the carboxy-terminal moiety of the leucine-rich domain which includes the leucine repeat, but not the Ala-Leu-Arg-Asp-X-Val sequences shared with each of the members of the *myc* gene family.

3. Expression of the mouse *vav* proto-oncogene

It has been previously shown that the human *vav* proto-oncogene is specifically expressed in cells of

hematopoietic origin regardless of their differentiation lineage [Katzav, S. et al., supra] confirms this pattern of expression. As summarized in Table 1, *vav* gene transcripts were identified in hematopoietic cells of myeloid (macrophagederived 7.1.3 cell line), lymphoid (MOPC 315 plasmacytoma and A20 B-lymphocyte cell lines) and erythroid (Friend erythroleukemia cells, F412B2 clone) origin. The levels of *vav* gene expression in undifferentiated mouse F412B2 cells were comparable to those present in the differentiated erythroid-like cells obtained by treatment of F412B2 cells with DMSO or HMBA. Similar results were obtained when human HEL and HL60 cells were induced to differentiate along different hematopoietic lineages [Katzav, S. et al., supra].

Northern blot analysis of RNA isolated from mouse fibroblastic cell lines failed to reveal detectable levels of *vav* gene expression (Table 1). These results were independent of the proliferative state of the cells since neither quiescent or serum-stimulated BALB3T3 cells possessed detectable *vav* gene transcripts. Similarly, *vav* gene expression was not found to correlate with the tumorigenic state of the cell since neither non-tumorigenic NIH3T3 cells or tumorigenic NIH3T3-derived ψ2 cells expressed detectable *vav* gene sequences (Table 1).

To determine the pattern of expression of the *vav* proto-oncogene *in vivo*, RNAs were isolated from various mouse tissues and submitted to Northern blot analysis. *vav* gene transcripts were observed in spleen and lung tissues but not in brain, heart, intestine, muscle, ovaries or testes (Figure 3). Expression of the *vav* gene in spleen cells indicates that this locus is expressed in hematopoetic cells *in vivo*. The presence of *vav* gene transcripts in lung raises the possibility that this gene may also be expressed in non-hematopietic cell types. However, lungs are known to contain high levels of infiltrating macrophages that may account for the results depicted in Figure 3.

4. Identification of the mouse *vav* proto-oncogene product

To identify the product of a mouse *vav* photo-oncogene, rabbits were immunized with a peptide whose sequence corresponded to that of an amphilic region conserved in a mouse and human *vav* gene proteins (amino acid residues 576 to 589 of Figure 2) [see SEQ. ID NO: 2] . Immunoprecipitation of [^{35}S-methionine]-labeled extracts of PAb280, a mouse B-cell hybridoma and PMMI, a mouse T-cell hybridoma with this anti-*vav* peptide antiserum revealed various polypeptides ranging in size between 75,000 and 105,000 daltons. The most intense band corresponded to a protein of about 95,000 daltons, a size that corresponds well with that expected for the *vav* gene product.

To establish whether this 95,000 dalton polypeptide was indeed the product of a mouse *vav* gene, an expression plasmid was generated by subcloning the entire cDNA insert of pMB24 into pMEX, an eukaryoticexpression vector [Martin-Zanca, D. et al., Mol. Cell Biol. 9, 24-33 (1989)]. The resulting plasmid, designated pJC11, was co-transfected into NIH3T3 cells with pSV*neo* and colonies of G418-resistant cells were selected for immunoprecipitation analysis. As illustrated in Figures 4C and D, cells transfected with pJC11 DNA expressed a 95,000 dalton protein indistinguishable from that present in mouse pAB280 and PMMI hybridoma cell lines. Moreover, immunoprecipitation of this 95,000 dalton protein was specifically blocked by preincubation with the immunizing peptide (Figure 4D). These results indicate that p95*vav* is the product of a mouse *vav* proto-oncogene.

Immunoprecipitation analysis of either hematopoietic cells or *vav*-transfected NIH3T3 clones consistently revealed a mior protein species that migrates as a diffuse band of about 105,000 daltons. Immunoprecipitation of this protein could be specifically blocked by competition with the immunizing peptide. Whether this protein represents a modified form of p95^{vav} or a different protein able to complex with the *vav* gene product awaits further biochemical characterization.

5. Malignant activation of the *vav* proto-oncogene

Transfection of NIH3T3 cells with pJC11 DNA, an expression plasmid carrying a mouse *vav* proto-oncogene, did not revealed significant levels of morphologic transformation (Figure 5). These results suggest that the transforming properties of the *vav* oncogene might be due to the absence of the *myc*-related amino-terminal domain and/or to the presence of the bacterial Tn5-derived sequences. To resolve this question, a truncated mouse *vav* gene was generated by deleting those nucleotide sequences of pJC11 DNA encompassed between the 5′ Sal I site of the pMEX multiple cloning site and a Nrul site that lies just upstream of the second in-frame ATG codon (nucleotides 301 to 303 in Figure 2) [see SEQ. ID NO: 1]. The resulting plasmid, desigated pJC12, codes for a truncated mouse *vav* protein that lacks 65 of the 67 amino-terminal residues absent in the human *vav* oncogene product (Katzav, S. et al., supra). Transfection of NIH3T3 cells with pJC12 DNA resulted in the appearance of about 3,000 foci of transformed cells per microgram of transfected DNA (Figure 5). Immunoprecipitation of [^{35}S-methionine]-labeled extracts of NIH3T3 cells transformed by pJC12 DNA with anti-*vav* peptide antibodies revealed expression of the expected 88,000 dalton protein (not shown). These results indicate

that truncation of the amino-terminal domain of a mouse *vav* proto-oncogene product can activate its transforming potential.

The transforming activity of pJC12 DNA is at least one order of magnitude lower than that of pSK27 DNA, the expression plasmid containing the human *vav* oncogene (Figure 5). To examine whether the Tn5-derived sequences also contribute to the transforming activity of the human *vav* oncogene, we generated pJC7, a pMEX-derived expression plasmid similar to pJC11 except that the *vav* sequences were of human origin. Since the longest human *vav* proto-oncogene cDNA clone ends four nucleotides short of the physiological ATG initiator codon, translation from pJC7 DNA is likely to start in the second in-frame ATG, the initiator codon used by pJC12. Transfection of NIH3T3 cells with pJC25 DNA resulted in the appearance of about 40,000 foci of transformed cells per microgram of transfected DNA, a transforming activity comparable to that of the human *vav* oncogene (Figure 5). These results indicate that the Tn5-derived sequences present in the human *vav* oncogene do not contribute to its transforming activity. Moreover, they demonstrate that truncation of the amino terminal domain of the *vav* gene product is sufficient to activate its neoplastic properties.

Finally, it was determined whether the human *vav* proto-oncogene possesses transforming activity. For this purpose, pJC7 was modified by adding the four nucleotides (ATGG) presumably missing in our human *vav* proto-oncogene cDNA clone. The resulting plasmid, pJC13, can only transform NIH3T3 cells with about 5% the activity of its parental clone, pJC7 (Figure 5). Analysis of NIH3T3 cells transformed by pJC13 DNA consistantly exhibited levels of expression of the normal $p95^{vav}$ proto-oncogene product 5- to 10-fold higher than those of the truncated *vav* protein found in cells transformed by pJC7 or pSK27 (Figure 6). These results indicate that the human *vav* proto-oncogene can only induce malignant transformation if overexpressed in NIH3T3 cells.

6. Identification of a second human *vav* oncogene: Mechanism of activation

A second human *vav* oncogene has been identified during the course of gene transfer experiments using DNAs isolated from mammary carcinomas (unpublished observations). To investigate whether this independently isolated *vav* oncogene also became activated by truncation of its amino terminus, two DNA probes were prepared by PCR-aided amplification of defined domains of the 5' region of pSK65, a human *vav* proto-oncogene cDNA clone (Katzav, S. et al., supra). The first probe is a 180 bp Eco RI-Hinc II DNA fragment which contains the 5' end of the human *vav* proto-oncogene cDNA clone, a region known to be absent in its transforming allele (Figure 7A). The second probe is a 575 bp Sac I-Pst I DNA fragment that corresponds to a region located 3' to the leucinerich domain and encompasses those sequences coding for the acidic region of the *vav* protein. As shown in Figure 7B, the 575 bp Sac I-Pst I probe recognized an internal 7 kbp Sac I fragment of normal human DNA which was also present in NIH3T3 cells transformed by the two independently isolated human *vav* oncogenes. In contrast, the most 5' 180 bp Eco RI-Hinc II probe only hybridized to normal human DNA (Figure 7A). These results indicate that a second human *vav* oncogene identified during gene transfer of mammary carcinoma DNA into NIH3T3 cells, has also lost those 5'sequences coding for the amino-terminal moiety of the *vav* leucine-rich region.

7. Contribution of the cysteine-rich domains to the biological activity of the *vav* gene products

The mouse and human *vav* gene products contain two structures that resemble metal binding domains. The first structure, located in residues 528-548 of a mouse $p95^{vav}$ protein (Figure 2), has a Cys-X_2-Cys-X_{13}-Cys-X_2-Cys sequence pattern. This motif has been previously found in several transcriptional activators such as the products of the adenovirus E1a, the yeast GAL 4 and various steroid receptor genes [Johnson, P.F. et al., Annu. Rev. Biochem. 58, 799-839 (1989)]. The second structure possesses a sequence pattern (His-X_2-Cys-S_6-Cys-X_2-His) that has not be previously described. The spacing of the cysteine residues along these putative metal binding structure (Cys-X_2-Cys-X_{13}-Cys-X_2-Cys-X_7-Cys-X_6-Cys), is also reminescent of the phorbol ester binding domain of protein kinase C [Ono, Y. et al., Proc. Natl. Acad. Sci. USA 86, 4868-4871 (1989)].

To test whether these structures are required for *vav* gene function, single point mutations were engineered in pJC12 and pJC7 DNAs that eliminated some of the conserved cystein and histidine-coding triplets. pJC12 and pJC7, two expression plasmids capable of inducing the malignant transformation of NIH3T3 cells, provide a reliable biological assay to measure *vav* gene activity. In order to verify the presence of the desired mutation, each of the mutated plasmids was submitted to nucleotide sequence analysis. In addition, these plasmids were transfected into NIH3T3 cells to verify that they directed the synthesis of the expected *vav* gene products (not shown).

As summarized in Table 2, replacement of the first or third cysteines of the metal binding-like domain by serine residues completely abolished the transforming activity of a mouse *vav* gene present in pJC12. Similar results were obtained when the first cysteine of the human *vav* gene was replaced by an arginine residue (Table

2). Finally, substitution of the histidine residue corresponding to the first position of a mouse His-X_2-Cys-X_6-Cys-X_2-His motif, also abolished *vav* transforming activity (Table 2). This histidine residue is one of five *vav* amino acids shared by the phorbol ester domains of protein kinase C. These results indicate that the overall structure of the cysteinerich domain of *vav* gene proteins is required for their biological function.

All publications and patents referred to in the present application are incorporated herein by reference to the same extent as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

TABLE 1

Expression of a mouse *vav* proto-oncogene in cells of murine origin[a]

CELL LINE	CELL TYPE	*vav* GENE EXPRESSION	REFERENCE
7.1.3	Macrophage	+	Baumbach et al., 1987[b]
MOPC 315	Plasmacytoma	+	ATCC TIB 23
A 20	B lymphocyte	+	ATCC TIB 208
F412B2	Erythroleukemia (undifferentiated)	+	Coppola and Cole, 1986[c]
F412B2 + HMBA	Erythroleukemia (differentiated)	+	
NIH3T3	Fibroblast (non-tumorigenic)	-	Jainchill et al., 1969[d]
NIH3T3/ψ-2	Fibroblast (tumorigenic)	-	Mann et al., 1983[e]
A31	Fibroblast (quiescent)	-	ATCC CCL 163
A31 + serum	Fibroblast (proliferating)	-	

[a] See legend to Figure 4 for experimental details.

[b] Baumbach, W.R. et al., Mol. Cell. Biol. 7, 664-671 (1987)

[c] Coppola, J.A. and Cole, M.D., Nature 320, 760-763 (1986)

[d] Jainchill, J.L. et al., J. Virol. 4, 549-553 (1969)

[e] Mann, R. et al., Cell 33, 153-159 (1983)

TABLE 2

Contribution of the cysteine-rich sequences to the Biological activity of *vav* gene proteins

PLASMID	SPECIES	CYSTEINE MOTIF[a]	TRANSFORMING ACTIVITY (ffu/μg DNA)[b]
pJC12	Mouse	$CX_2CX_{13}CX_2CX_4HX_2CX_6CX_2H$	450
pJC17	Mouse	$\underline{S}X_2CX_{13}CX_2CX_4HX_2CX_6CX_2H$	0
pJC18	Mouse	$CX_2CX_{13}\underline{S}X_2CX_4HX_2CX_6CX_2H$	0
pJC19	Mouse	$CX_2CX_{13}CX_2CX_4\underline{D}X_2CX_6CX_2H$	0
pJC5	Human	$CX_2CX_{13}CX_2CX_4HX_2CX_6CX_2H$	5,000
pJC15	Human	$\underline{R}X_2CX_{13}CX_2CX_4HX_2CX_6CX_2H$	0

[a] Cysteine motifs (residues 528 to 566) contain metal binding-like domains ($Cys-X_2-Cys-X_{13}-Cys-X_2-Cys$ and $His-X_2-Cys-X_6-Cys-X_2-His$) and putative phorbol ester binding regions ($Cys-X_2-Cya-X_{13}-Cys-X_7-Cys-X_6-Cys$). Substituted amino acid residues are bolded and underlined.

[b] pSK27 DNA (see Figure 6) used as positive control in this experiment yielded 5,000 ffu/μg DNA.

SEQUENCE LISTING

NUMBER OF SEQUENCES: 2

INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2793 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: N

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 14..2545
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GCCGGCAGCC ACC ATG GAG CTC TGG CGA CAG TGC ACC CAC TGG CTG ATC                49
               Met Glu Leu Trp Arg Gln Cys Thr His Trp Leu Ile
                 1               5                  10

CAG TGT CGG GTG CTG CCT CCC AGC CAC CGT GTG ACC TGG GAG GGG GCC              97
Gln Cys Arg Val Leu Pro Pro Ser His Arg Val Thr Trp Glu Gly Ala
          15                  20                  25

CAG GTG TGT GAG CTG GCA CAG GCA CTG CGG GAC GGT GTC CTC TTG TGC             145
Gln Val Cys Glu Leu Ala Gln Ala Leu Arg Asp Gly Val Leu Leu Cys
      30                  35                  40

CAA TTG CTT AAC AAC CTG CTT CCC CAG GCC ATT AAT CTT CGC GAG GTT             193
Gln Leu Leu Asn Asn Leu Leu Pro Gln Ala Ile Asn Leu Arg Glu Val
 45                  50                  55                  60

AAC TTG CGG CCC CAG ATG TCC CAG TTC CTT TGT CTT AAG AAC ATT CGA             241
Asn Leu Arg Pro Gln Met Ser Gln Phe Leu Cys Leu Lys Asn Ile Arg
                 65                  70                  75

ACC TTC CTG TCT ACT TGC TGT GAG AAG TTC GGC CTC AAG CGC AGT GAA             289
Thr Phe Leu Ser Thr Cys Cys Glu Lys Phe Gly Leu Lys Arg Ser Glu
             80                  85                  90

CTC TTT GAG GCT TTT GAC CTC TTC GAT GTG CAG GAC TTT GGA AAG GTC             337
Leu Phe Glu Ala Phe Asp Leu Phe Asp Val Gln Asp Phe Gly Lys Val
          95                 100                 105

ATC TAC ACC CTG TCT GCT CTG TCA TGG ACA CCC ATT GCC CAG AAC AAA             385
Ile Tyr Thr Leu Ser Ala Leu Ser Trp Thr Pro Ile Ala Gln Asn Lys
     110                 115                 120

GGA ATC ATG CCC TTC CCA ACA GAG GAC AGC GCT CTG AAC GAC GAA GAT             433
Gly Ile Met Pro Phe Pro Thr Glu Asp Ser Ala Leu Asn Asp Glu Asp
 125                 130                 135                 140

ATT TAC AGT GGC CTT TCA GAC CAG ATT GAT GAC ACC GCA GAG GAA GAC             481
Ile Tyr Ser Gly Leu Ser Asp Gln Ile Asp Asp Thr Ala Glu Glu Asp
                 145                 150                 155

GAG GAC CTT TAT GAC TGC GTG GAA AAT GAG GAG GCA GAG GGG GAC GAG             529
Glu Asp Leu Tyr Asp Cys Val Glu Asn Glu Glu Ala Glu Gly Asp Glu
             160                 165                 170

ATC TAC GAG GAC CTA ATG CGC TTG GAG TCG GTG CCT ACG CCA CCC AAG             577
Ile Tyr Glu Asp Leu Met Arg Leu Glu Ser Val Pro Thr Pro Pro Lys
          175                 180                 185

ATG ACA GAG TAT GAT AAG CGC TGC TGC TGC CTG CGG GAG ATC CAG CAG             625
Met Thr Glu Tyr Asp Lys Arg Cys Cys Cys Leu Arg Glu Ile Gln Gln
     190                 195                 200

ACG GAG GAG AAG TAT ACA GAC ACA CTG GGC TCC ATC CAG CAG CAC TTC             673
Thr Glu Glu Lys Tyr Thr Asp Thr Leu Gly Ser Ile Gln Gln His Phe
 205                 210                 215                 220

ATG AAG CCT CTG CAG CGA TTC CTT AAG CCT CAA GAC ATG GAG ACC ATC             721
Met Lys Pro Leu Gln Arg Phe Leu Lys Pro Gln Asp Met Glu Thr Ile
                 225                 230                 235

TTT GTC AAC ATT GAG GAG CTG TTC TCT GTG CAT ACC CAC TTC TTA AAG             769
Phe Val Asn Ile Glu Glu Leu Phe Ser Val His Thr His Phe Leu Lys
             240                 245                 250

GAA CTG AAG GAT GCC CTG GCT GGC CCG GGA GCA ACA ACA CTG TAT CAG             817
Glu Leu Lys Asp Ala Leu Ala Gly Pro Gly Ala Thr Thr Leu Tyr Gln
          255                 260                 265
```

```
GTC TTC ATC AAG TAC AAG GAG AGG TTC CTG GTT TAT GGC CGT TAT TGC          865
Val Phe Ile Lys Tyr Lys Glu Arg Phe Leu Val Tyr Gly Arg Tyr Cys
    270                 275                 280

AGT CAG GTG GAG TCA GCC AGC AAG CAC TTG GAT CAA GTG GCC ACA GCA          913
Ser Gln Val Glu Ser Ala Ser Lys His Leu Asp Gln Val Ala Thr Ala
285                 290                 295                 300

CGG GAG GAT GTG CAG ATG AAG CTG GAG GAA TGT TCT CAA AGA GCT AAC          961
Arg Glu Asp Val Gln Met Lys Leu Glu Glu Cys Ser Gln Arg Ala Asn
                305                 310                 315

AAT GGC CGA TTC ACC CTA CGG TCT GCT GAT GGT ACC TAT GCA GCG GGT         1009
Asn Gly Arg Phe Thr Leu Arg Ser Ala Asp Gly Thr Tyr Ala Ala Gly
            320                 325                 330

GCT GAA GTA CCA CCT CCT TCT CCA GGA GCT AGT GAA ACA CAC ACA GGA         1057
Ala Glu Val Pro Pro Pro Ser Pro Gly Ala Ser Glu Thr His Thr Gly
        335                 340                 345

TGC TAC AGA GAA GGA GAA CTG CGG TTG GCC CTG GAC GCC ATG AGG GAC         1105
Cys Tyr Arg Glu Gly Glu Leu Arg Leu Ala Leu Asp Ala Met Arg Asp
    350                 355                 360

CTG GCA CAG TGC GTG AAC GAG GTC AAG AGG GAC AAT GAA ACC CTA CGG         1153
Leu Ala Gln Cys Val Asn Glu Val Lys Arg Asp Asn Glu Thr Leu Arg
365                 370                 375                 380

CAG ATC ACA AAC TTT CAG CTG TCC ATT GAG AAC CTG GAC CAG TCT CTG         1201
Gln Ile Thr Asn Phe Gln Leu Ser Ile Glu Asn Leu Asp Gln Ser Leu
                385                 390                 395

GCT AAC TAT GGC CGG CCC AAG ATT GAC GGT GAG CTC AAG ATT ACC TCA         1249
Ala Asn Tyr Gly Arg Pro Lys Ile Asp Gly Glu Leu Lys Ile Thr Ser
            400                 405                 410

GTG GAG CGT CGC TCA AAG ACA GAC AGG TAT GCC TTC CTG CTG GAC AAA         1297
Val Glu Arg Arg Ser Lys Thr Asp Arg Tyr Ala Phe Leu Leu Asp Lys
        415                 420                 425

GCA CTG CTC ATC TGT AAA CGC CGC GGG GAC TCT TAC GAC CTC AAA GCC         1345
Ala Leu Leu Ile Cys Lys Arg Arg Gly Asp Ser Tyr Asp Leu Lys Ala
    430                 435                 440

TCG GTG AAC TTG CAC AGC TTC CAA GTT TCA GAT GAC TCC TCC GGG GAG         1393
Ser Val Asn Leu His Ser Phe Gln Val Ser Asp Asp Ser Ser Gly Glu
445                 450                 455                 460

CGA GAC AAC AAG AAG TGG AGC CAT ATG TTC CTT CTG ATT GAG GAT CAA         1441
Arg Asp Asn Lys Lys Trp Ser His Met Phe Leu Leu Ile Glu Asp Gln
                465                 470                 475
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GGC GCC CAG GGC TAT GAG CTG TTC TTC AAG ACT CGG GAG CTG AAG AAG      1489
Gly Ala Gln Gly Tyr Glu Leu Phe Phe Lys Thr Arg Glu Leu Lys Lys
            480                 485                 490

AAG TGG ATG GAA CAG TTC GAA ATG GCC ATC TCC AAC ATT TAC CCA GAG      1537
Lys Trp Met Glu Gln Phe Glu Met Ala Ile Ser Asn Ile Tyr Pro Glu
        495                 500                 505

AAT GCT ACA GCC AAT GGG CAT GAT TTT CAG ATG TTC TCC TTT GAG GAG      1585
Asn Ala Thr Ala Asn Gly His Asp Phe Gln Met Phe Ser Phe Glu Glu
    510                 515                 520

ACC ACT TCC TGC AAG GCC TGC CAG ATG TTA CTC AGA GGC ACA TTC TAC      1633
Thr Thr Ser Cys Lys Ala Cys Gln Met Leu Leu Arg Gly Thr Phe Tyr
525                 530                 535                 540

CAG GGA TAT CGC TGT TAC AGG TGC CGG GCA CCT GCA CAC AAG GAG TGT      1681
Gln Gly Tyr Arg Cys Tyr Arg Cys Arg Ala Pro Ala His Lys Glu Cys
                545                 550                 555

CTG GGG AGA GTG CCT CCC TGT GGT CGC CAT GGG CAA GAT TTC GCA GGA      1729
Leu Gly Arg Val Pro Pro Cys Gly Arg His Gly Gln Asp Phe Ala Gly
            560                 565                 570

ACC ATG AAG AAG GAC AAG CTC CAT CGA AGG GCC CAG GAC AAG AAA AGG      1777
Thr Met Lys Lys Asp Lys Leu His Arg Arg Ala Gln Asp Lys Lys Arg
        575                 580                 585

AAT GAA TTG GGT CTG CCT AAG ATG GAA GTG TTT CAG GAA TAC TAT GGG      1825
Asn Glu Leu Gly Leu Pro Lys Met Glu Val Phe Gln Glu Tyr Tyr Gly
    590                 595                 600

ATC CCA CCA CCA CCT GGA GCC TTT GGG CCA TTT TTA CGG CTC AAC CCT      1873
Ile Pro Pro Pro Pro Gly Ala Phe Gly Pro Phe Leu Arg Leu Asn Pro
605                 610                 615                 620

GGG GAC ATT GTG GAG CTC ACT AAG GCA GAG GCT GAG CAC AAC TGG TGG      1921
Gly Asp Ile Val Glu Leu Thr Lys Ala Glu Ala Glu His Asn Trp Trp
                625                 630                 635

GAG GGA AGG AAT ACT GCT ACA AAT GAA GTC GGC TGG TTT CCC TGT AAC      1969
Glu Gly Arg Asn Thr Ala Thr Asn Glu Val Gly Trp Phe Pro Cys Asn
            640                 645                 650

AGA GTG CAT CCC TAT GTC CAC GGC CCT CCT CAG GAC CTG TCT GTG CAT      2017
Arg Val His Pro Tyr Val His Gly Pro Pro Gln Asp Leu Ser Val His
        655                 660                 665
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CTC TGG TAT GCG GGC CCT ATG GAA CGA GCA GGC GCT GAG GGC ATC CTC      2065
Leu Trp Tyr Ala Gly Pro Met Glu Arg Ala Gly Ala Glu Gly Ile Leu
    670                 675                 680

ACC AAC CGT TCT GAT GGG ACC TAT CTG GTG CGG CAG AGG GTG AAA GAT      2113
Thr Asn Arg Ser Asp Gly Thr Tyr Leu Val Arg Gln Arg Val Lys Asp
685                 690                 695                 700

ACA GCG GAG TTC GCC ATC AGC ATT AAG TAT AAC GTG GAG GTC AAG CAT      2161
Thr Ala Glu Phe Ala Ile Ser Ile Lys Tyr Asn Val Glu Val Lys His
                705                 710                 715

ATT AAA ATC ATG ACG TCA GAG GGG TTG TAC CGG ATC ACA GAG AAG AAG      2209
Ile Lys Ile Met Thr Ser Glu Gly Leu Tyr Arg Ile Thr Glu Lys Lys
            720                 725                 730

GCT TTC CGG GGC CTT CTG GAA CTG GTA GAG TTT TAT CAG CAG AAT TCC      2257
Ala Phe Arg Gly Leu Leu Glu Leu Val Glu Phe Tyr Gln Gln Asn Ser
        735                 740                 745

CTC AAA GAT TGC TTC AAG TCG TTG GAC ACC ACC TTG CAG TTT CCT TAT      2305
Leu Lys Asp Cys Phe Lys Ser Leu Asp Thr Thr Leu Gln Phe Pro Tyr
    750                 755                 760

AAG GAA CCT GAG AGG AGA GCC ATC AGC AAG CCA CCA GCT GGA AGC ACC      2353
Lys Glu Pro Glu Arg Arg Ala Ile Ser Lys Pro Pro Ala Gly Ser Thr
765                 770                 775                 780

AAG TAT TTT GGC ACT GCC AAA GCC CGC TAC GAC TTC TGT GCC CGG GAC      2401
Lys Tyr Phe Gly Thr Ala Lys Ala Arg Tyr Asp Phe Cys Ala Arg Asp
                785                 790                 795

AGG TCG GAA CTG TCC CTT AAG GAG GGT GAT ATC ATC AAG ATC CTC AAT      2449
Arg Ser Glu Leu Ser Leu Lys Glu Gly Asp Ile Ile Lys Ile Leu Asn
            800                 805                 810

AAG AAG GGA CAG CAA GGC TGG TGG CGT GGG GAG ATC TAC GGC CGG ATC      2497
Lys Lys Gly Gln Gln Gly Trp Trp Arg Gly Glu Ile Tyr Gly Arg Ile
        815                 820                 825

GGC TGG TTC CCT TCT AAC TAT GTG GAG GAA GAC TAT TCC GAA TAT TGC      2545
Gly Trp Phe Pro Ser Asn Tyr Val Glu Glu Asp Tyr Ser Glu Tyr Cys
    830                 835                 840

TGAGCCTGGT GCCCTGTAGG ACACAGAGAG AGGCAGATGA AGGCTGAGCC CAGGATGCTA    2605

GCAGGGTTGA GGGGCCATGA ACTGTCCTCA CCACGGAGGA TCTGGATGCG TGCAGATGGC    2665

TAGTGGCCAG CTGGCAGGGT TCCCAGGATA AAGCCCAGAG ATGCGTAATT TATAACACAC    2725

TGATTTTCTC CAGTCCTCCA CGAAAGGTGG GGCTTGAGGC AACTGATTCT AATAAAGTGA    2785

GGAGAGCA                                                             2793
```

INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 844 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Glu Leu Trp Arg Gln Cys Thr His Trp Leu Ile Gln Cys Arg Val
 1               5                  10                  15

Leu Pro Pro Ser His Arg Val Thr Trp Glu Gly Ala Gln Val Cys Glu
            20                  25                  30

Leu Ala Gln Ala Leu Arg Asp Gly Val Leu Leu Cys Gln Leu Leu Asn
        35                  40                  45

Asn Leu Leu Pro Gln Ala Ile Asn Leu Arg Glu Val Asn Leu Arg Pro
    50                  55                  60

Gln Met Ser Gln Phe Leu Cys Leu Lys Asn Ile Arg Thr Phe Leu Ser
65                  70                  75                  80

Thr Cys Cys Glu Lys Phe Gly Leu Lys Arg Ser Glu Leu Phe Glu Ala
                85                  90                  95

Phe Asp Leu Phe Asp Val Gln Asp Phe Gly Lys Val Ile Tyr Thr Leu
            100                 105                 110

Ser Ala Leu Ser Trp Thr Pro Ile Ala Gln Asn Lys Gly Ile Met Pro
        115                 120                 125

Phe Pro Thr Glu Asp Ser Ala Leu Asn Asp Glu Asp Ile Tyr Ser Gly
    130                 135                 140

Leu Ser Asp Gln Ile Asp Asp Thr Ala Glu Glu Asp Glu Asp Leu Tyr
145                 150                 155                 160

Asp Cys Val Glu Asn Glu Glu Ala Glu Gly Asp Glu Ile Tyr Glu Asp
                165                 170                 175

Leu Met Arg Leu Glu Ser Val Pro Thr Pro Pro Lys Met Thr Glu Tyr
            180                 185                 190

Asp Lys Arg Cys Cys Cys Leu Arg Glu Ile Gln Gln Thr Glu Glu Lys
        195                 200                 205
```

Tyr Thr Asp Thr Leu Gly Ser Ile Gln Gln His Phe Met Lys Pro Leu
210 215 220

Gln Arg Phe Leu Lys Pro Gln Asp Met Glu Thr Ile Phe Val Asn Ile
225 230 235 240

Glu Glu Leu Phe Ser Val His Thr His Phe Leu Lys Glu Leu Lys Asp
245 250 255

Ala Leu Ala Gly Pro Gly Ala Thr Thr Leu Tyr Gln Val Phe Ile Lys
260 265 270

Tyr Lys Glu Arg Phe Leu Val Tyr Gly Arg Tyr Cys Ser Gln Val Glu
275 280 285

Ser Ala Ser Lys His Leu Asp Gln Val Ala Thr Ala Arg Glu Asp Val
290 295 300

Gln Met Lys Leu Glu Glu Cys Ser Gln Arg Ala Asn Asn Gly Arg Phe
305 310 315 320

Thr Leu Arg Ser Ala Asp Gly Thr Tyr Ala Ala Gly Ala Glu Val Pro
325 330 335

Pro Pro Ser Pro Gly Ala Ser Glu Thr His Thr Gly Cys Tyr Arg Glu
340 345 350

Gly Glu Leu Arg Leu Ala Leu Asp Ala Met Arg Asp Leu Ala Gln Cys
355 360 365

Val Asn Glu Val Lys Arg Asp Asn Glu Thr Leu Arg Gln Ile Thr Asn
370 375 380

Phe Gln Leu Ser Ile Glu Asn Leu Asp Gln Ser Leu Ala Asn Tyr Gly
385 390 395 400

Arg Pro Lys Ile Asp Gly Glu Leu Lys Ile Thr Ser Val Glu Arg Arg
405 410 415

Ser Lys Thr Asp Arg Tyr Ala Phe Leu Leu Asp Lys Ala Leu Leu Ile
420 425 430

Cys Lys Arg Arg Gly Asp Ser Tyr Asp Leu Lys Ala Ser Val Asn Leu
435 440 445

His Ser Phe Gln Val Ser Asp Asp Ser Ser Gly Glu Arg Asp Asn Lys
450 455 460

Lys Trp Ser His Met Phe Leu Leu Ile Glu Asp Gln Gly Ala Gln Gly
465 470 475 480

Tyr Glu Leu Phe Phe Lys Thr Arg Glu Leu Lys Lys Lys Trp Met Glu
485 490 495

Gln Phe Glu Met Ala Ile Ser Asn Ile Tyr Pro Glu Asn Ala Thr Ala
500 505 510

```
Asn Gly His Asp Phe Gln Met Phe Ser Phe Glu Glu Thr Thr Ser Cys
        515                 520                 525

Lys Ala Cys Gln Met Leu Leu Arg Gly Thr Phe Tyr Gln Gly Tyr Arg
    530                 535                 540

Cys Tyr Arg Cys Arg Ala Pro Ala His Lys Glu Cys Leu Gly Arg Val
545                 550                 555                 560

Pro Pro Cys Gly Arg His Gly Gln Asp Phe Ala Gly Thr Met Lys Lys
                565                 570                 575

Asp Lys Leu His Arg Arg Ala Gln Asp Lys Lys Arg Asn Glu Leu Gly
            580                 585                 590

Leu Pro Lys Met Glu Val Phe Gln Glu Tyr Tyr Gly Ile Pro Pro Pro
        595                 600                 605

Pro Gly Ala Phe Gly Pro Phe Leu Arg Leu Asn Pro Gly Asp Ile Val
    610                 615                 620

Glu Leu Thr Lys Ala Glu Ala Glu His Asn Trp Trp Glu Gly Arg Asn
625                 630                 635                 640

Thr Ala Thr Asn Glu Val Gly Trp Phe Pro Cys Asn Arg Val His Pro
                645                 650                 655

Tyr Val His Gly Pro Pro Gln Asp Leu Ser Val His Leu Trp Tyr Ala
            660                 665                 670

Gly Pro Met Glu Arg Ala Gly Ala Glu Gly Ile Leu Thr Asn Arg Ser
        675                 680                 685

Asp Gly Thr Tyr Leu Val Arg Gln Arg Val Lys Asp Thr Ala Glu Phe
    690                 695                 700

Ala Ile Ser Ile Lys Tyr Asn Val Glu Val Lys His Ile Lys Ile Met
705                 710                 715                 720

Thr Ser Glu Gly Leu Tyr Arg Ile Thr Glu Lys Lys Ala Phe Arg Gly
                725                 730                 735

Leu Leu Glu Leu Val Glu Phe Tyr Gln Gln Asn Ser Leu Lys Asp Cys
            740                 745                 750

Phe Lys Ser Leu Asp Thr Thr Leu Gln Phe Pro Tyr Lys Glu Pro Glu
        755                 760                 765

Arg Arg Ala Ile Ser Lys Pro Pro Ala Gly Ser Thr Lys Tyr Phe Gly
    770                 775                 780

Thr Ala Lys Ala Arg Tyr Asp Phe Cys Ala Arg Asp Arg Ser Glu Leu
785                 790                 795                 800

Ser Leu Lys Glu Gly Asp Ile Ile Lys Ile Leu Asn Lys Lys Gly Gln
                805                 810                 815

Gln Gly Trp Trp Arg Gly Glu Ile Tyr Gly Arg Ile Gly Trp Phe Pro
            820                 825                 830

Ser Asn Tyr Val Glu Glu Asp Tyr Ser Glu Tyr Cys
        835                 840
```

Claims

1. An isolated nucleic acid molecule comprising a nucleic acid sequence coding for all or part of a mouse

vav proto-oncogene protein or for a modified mouse *vav* proto-oncogene protein.

2. The nucleic acid molecule according to Claim 1 which is a DNA molecule and wherein the nucleic acid sequence is a DNA sequence.

3. The DNA molecule according to Claim 2 wherein the DNA sequence has the nucleotide sequence substantially as shown in Figure 2 [SEQ. ID No: 1].

4. The DNA molecule according to Claim 2 wherein the DNA sequence has part of the nucleotide sequence substantially as shown in Figure 2 [SEQ. ID No: 1].

5. A DNA molecule having a DNA sequence which is complementary to the DNA sequence according to Claims 3 or 4.

6. An expression vector comprising a DNA sequence coding for all or part of a mouse *vav* proto-oncogene protein or for a modified mouse *vav* proto-oncogene protein.

7. The expression vector according to Claim 6 comprising one or more control DNA sequences capable of directing the replication and/or the expression of and operatively linked to the DNA sequence coding for all or part of a mouse *vav* proto-oncogene protein or for a modified mouse *vav* proto-oncogene protein.

8. The expression vector according to Claim 6 wherein the DNA sequence coding for all or part of a mouse *vav* proto-oncogene protein or for a modified mouse *vav* proto-oncogene protein has the nucleotide sequence substantially as shown in Figure 2 [SEQ. ID No: 1].

9. The expression vector according to Claim 6 wherein the DNA sequence coding for all or part of a mouse *vav* proto-oncogene protein or for a modified mouse *vav* proto-oncogene protein has part of the nucleotide sequence substantially as shown in Figure 2 [SEQ. ID NO: 1].

10. The expression vector according to Claim 6 designated pMB24.

11. An expression vector having the identifying characteristics of the expression vector according to Claim 10.

12. A prokaryotic or eukaryotic host cell containing the expression vector according to any one of Claims 6 to 11.

13. A method for producing a polypeptide molecule which comprises all or part of a mouse *vav* proto-oncogene protein or a modified mouse *vav* proto-oncogene protein comprising culturing a host cell according to Claim 12 under conditions permitting expression of the polypeptide molecule.

14. A method for detecting a nucleic acid sequence coding for all or part of a mouse *vav* proto-oncogene protein or a related nucleic acid sequence comprising contacting the nucleic acid sequence with a detectable marker which binds specifically to at least part of the nucleic acid sequence, and detecting the marker so bound, the presence of bound marker indicating the presence of the nucleic acid sequence.

15. The method according to Claim 14 wherein the nucleic acid sequence is a DNA sequence.

16. The method according to Claim 14 wherein the nucleic acid sequence is an RNA sequence.

17. The method according to Claim 15 wherein the DNA sequence has the nucleotide sequence substantially as shown in Figure 2 [SEQ. ID No: 1].

18. The method according to Claim 15 wherein the DNA sequence has part of the nucleotide sequence substantially as shown in Figure 2 [SEQ. ID No: 1].

19. The method according to any one of claims 14 to 18 wherein the detectable marker is a nucleotide sequence complementary to at least a portion of the nucleic acid sequence.

20. The method acording to Claim 19 wherein the nucleotide sequence is a complementary DNA sequence.

21. The method according to Claim 15 wherein the DNA sequence is a genomic DNA sequence.

22. The method according to Claim 16 wherein the RNA sequence is a messenger RNA sequence.

23. An isolated polypeptide molecule comprising all or part of a mouse *vav* proto-oncogene protein or a modified mouse *vav* proto-oncogene protein.

24. An isolated polypeptide molecule encoded by the DNA sequence according to Claim 2.

25. The polypeptide molecule according to Claim 23 having the amino acid sequence substantially as shown in Figure 2 [SEQ. ID NO: 2].

26. The polypeptide molecule according to Claim 23 having part of the amino acid sequence substantially as shown in Figure 2 [SEQ. ID NO: 2].

FIG. 1

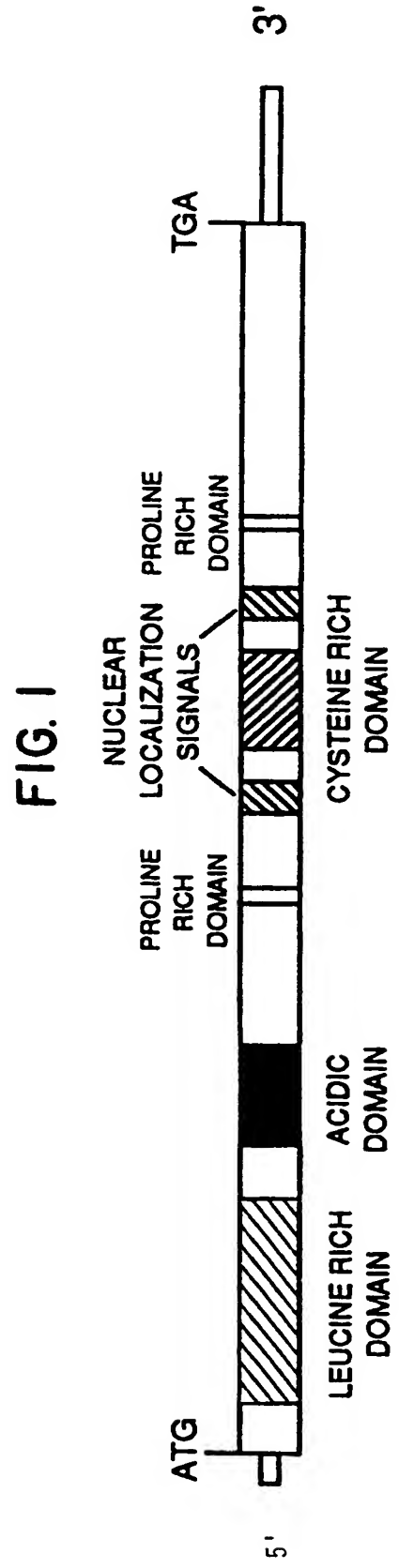
3'
TGA
PROLINE RICH DOMAIN
NUCLEAR LOCALIZATION SIGNALS
CYSTEINE RICH DOMAIN
PROLINE RICH DOMAIN
ACIDIC DOMAIN
LEUCINE RICH DOMAIN
ATG
5'

FIG.2A

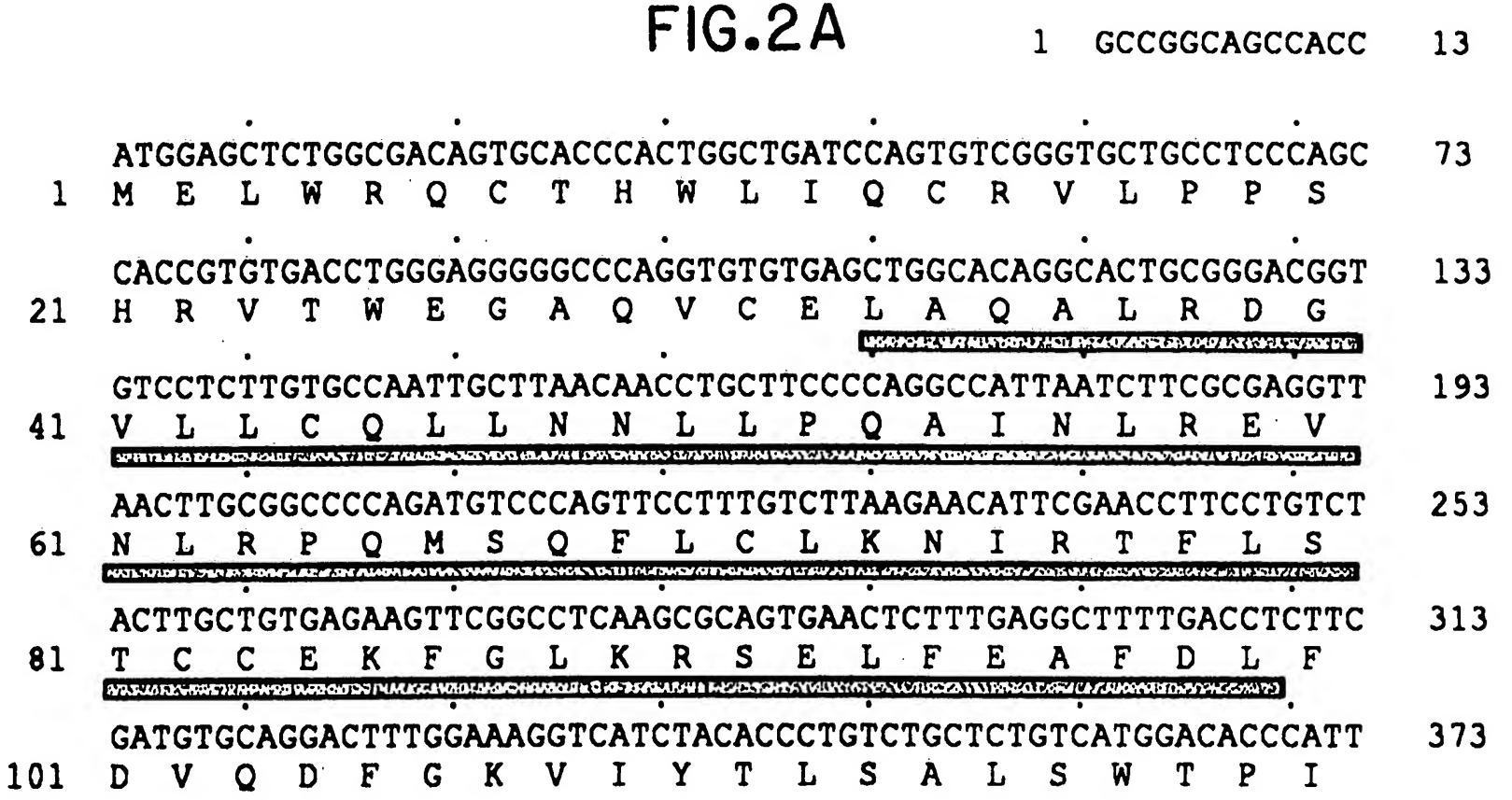

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FIG.2B

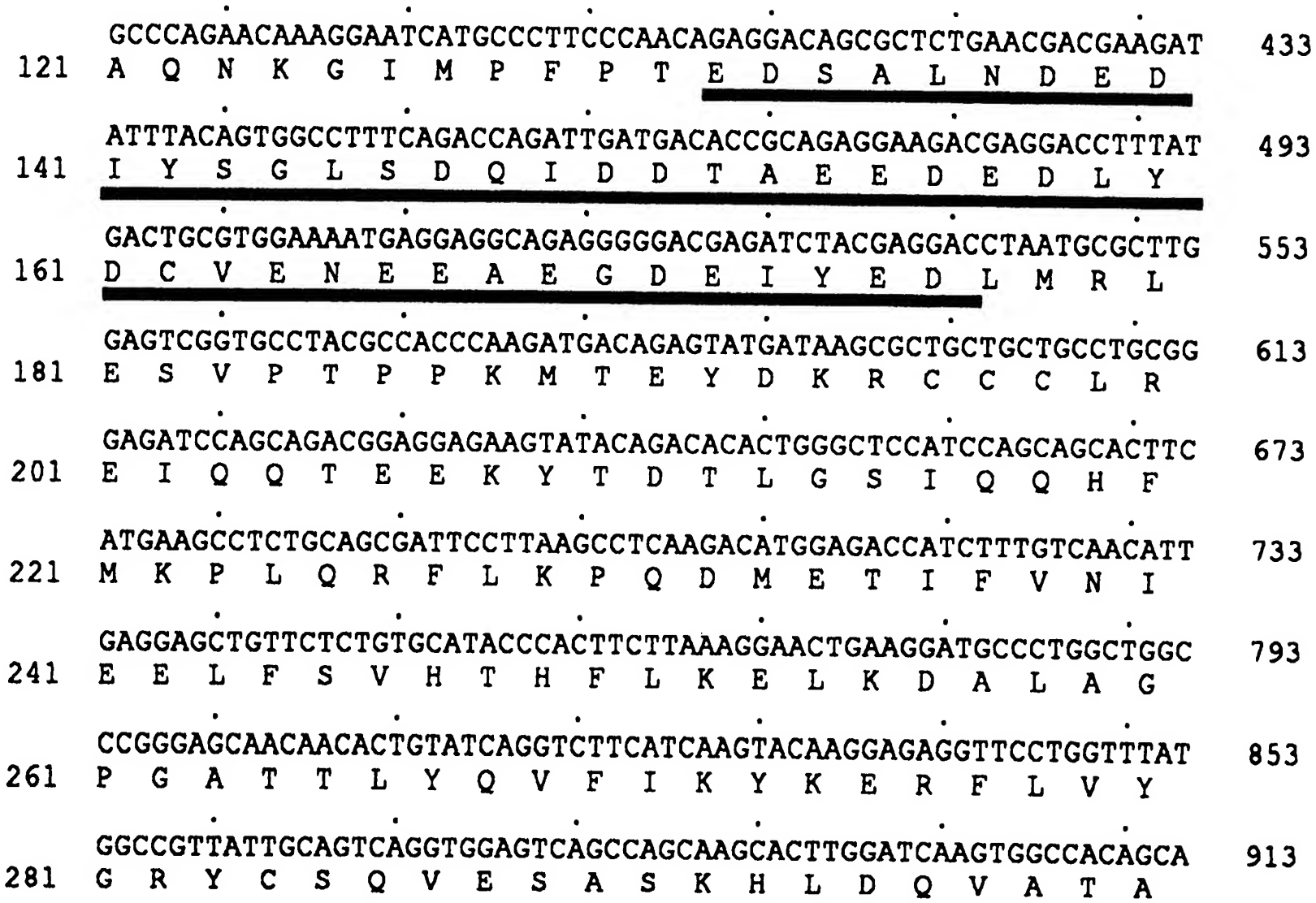

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FIG.2C

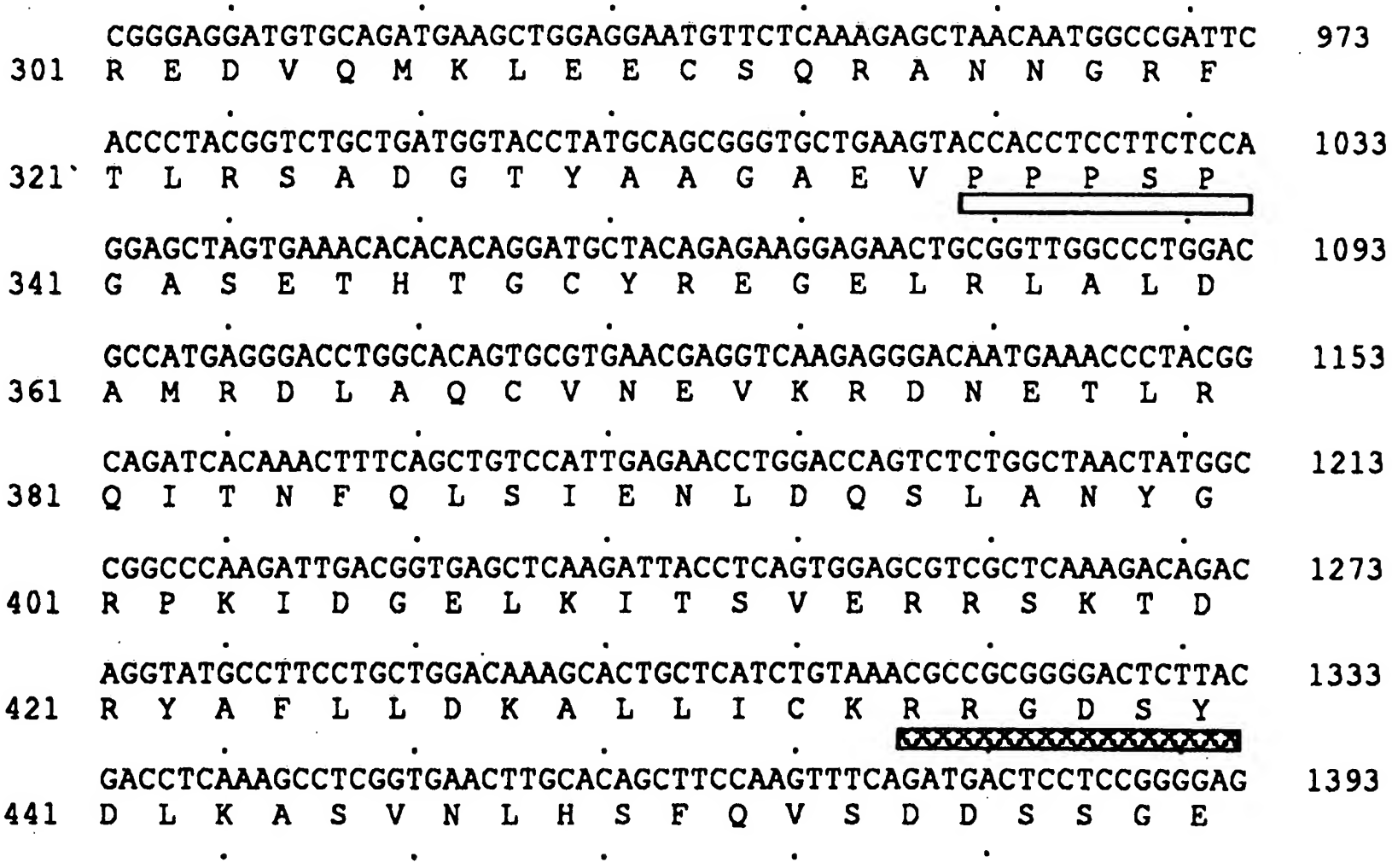

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FIG.2D

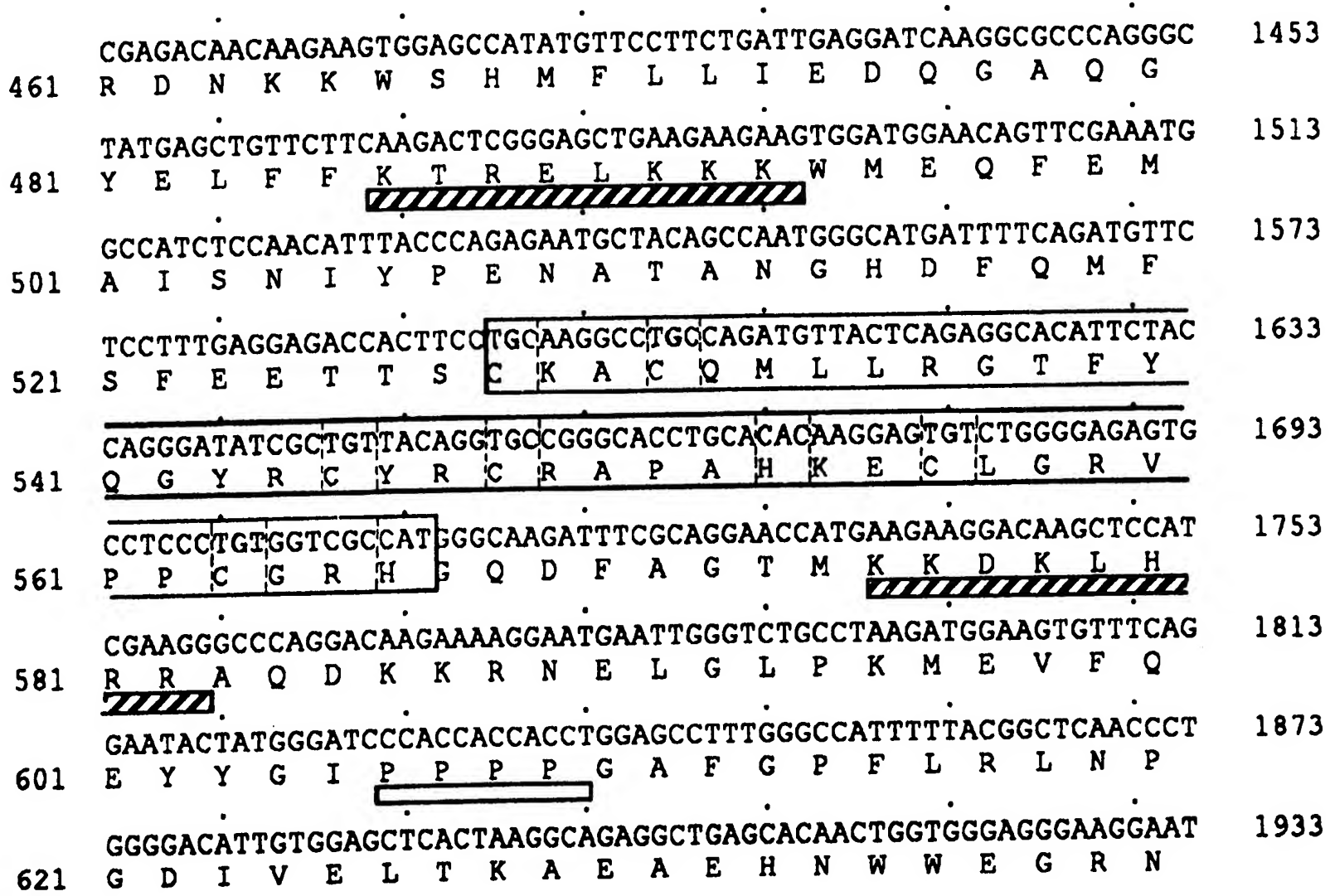

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FIG.2E

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     ACTGCTACAAATGAAGTCGGCTGGTTTCCCTGTAACAGAGTGCATCCCTATGTCCACGGC   1993
641  T  A  T  N  E  V  G  W  F  P  C  N  R  V  H  P  Y  V  H  G

     CCTCCTCAGGACCTGTCTGTGCATCTCTGGTATGCGGGCCCTATGGAACGAGCAGGCGCT   2053
661  P  P  Q  D  L  S  V  H  L  W  Y  A  G  P  M  E  R  A  G  A

     GAGGGCATCCTCACCAACCGTTCTGATGGGACCTATCTGGTGCGGCAGAGGGTGAAAGAT   2113
681  E  G  I  L  T  N  R  S  D  G  T  Y  L  V  R  Q  R  V  K  D

     ACAGCGGAGTTCGCCATCAGCATTAAGTATAACGTGGAGGTCAAGCATATTAAAATCATG   2173
701  T  A  E  F  A  I  S  I  K  Y  N  V  E  V  K  H  I  K  I  M

     ACGTCAGAGGGGTTGTACCGGATCACAGAGAAGAAGGCTTTCCGGGGCCTTCTGGAACTG   2233
721  T  S  E  G  L  Y  R  I  T  E  K  K  A  F  R  G  L  L  E  L

     GTAGAGTTTTATCAGCAGAATTCCCTCAAAGATTGCTTCAAGTCGTTGGACACCACCTTG   2293
741  V  E  F  Y  Q  Q  N  S  L  K  D  C  F  K  S  L  D  T  T  L

     CAGTTTCCTTATAAGGAACCTGAGAGGAGAGCCATCAGCAAGCCACCAGCTGGAAGCACC   2353
761  Q  F  P  Y  K  E  P  E  R  R  A  I  S  K  P  P  A  G  S  T
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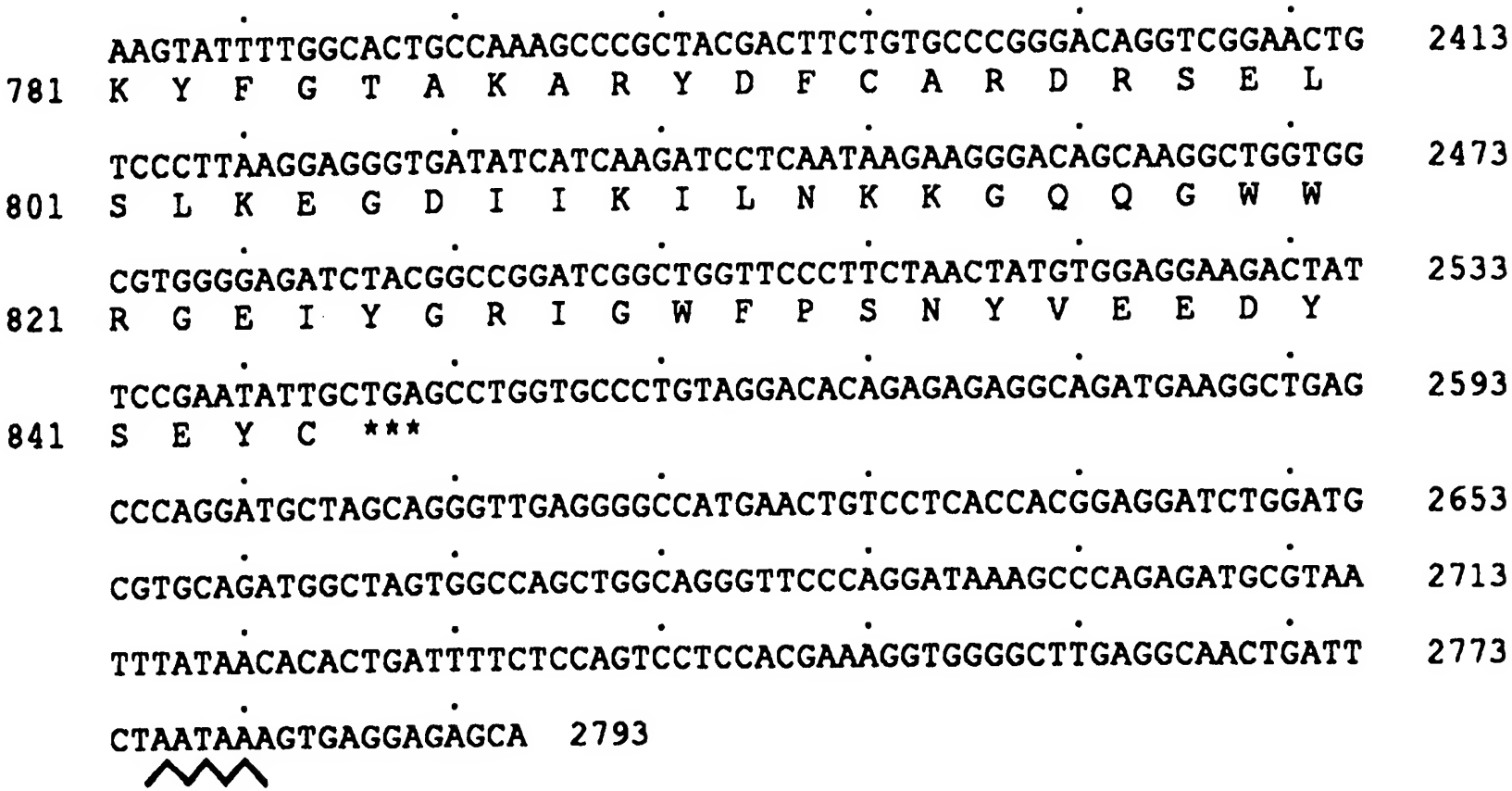
FIG.2F



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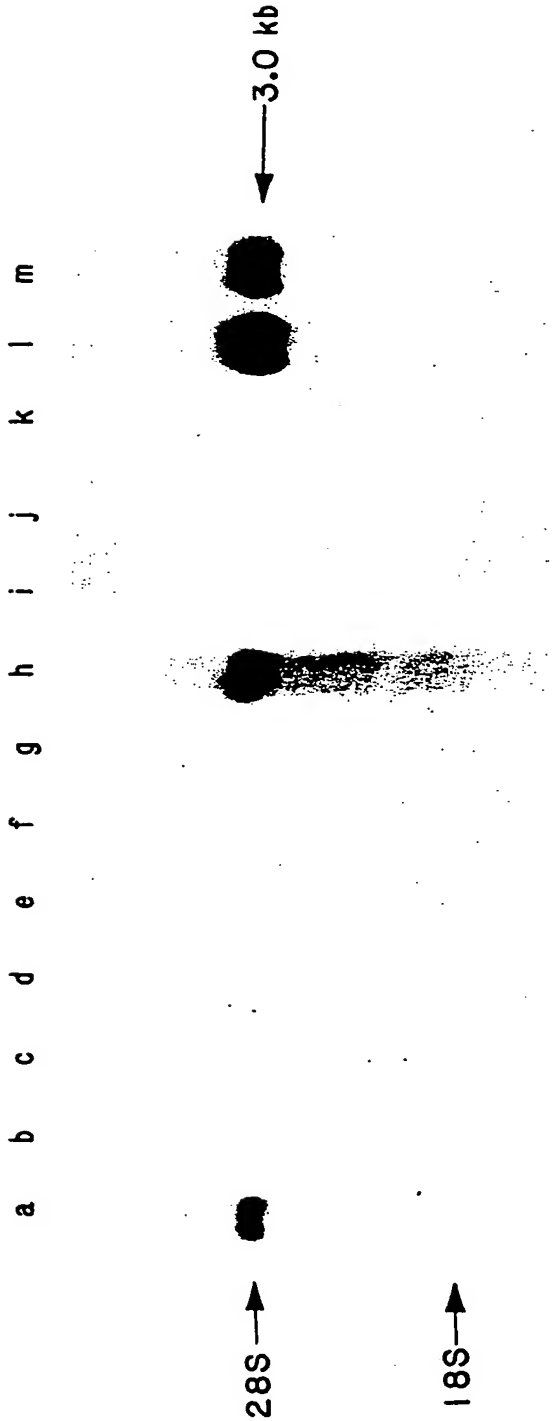


FIG. 3

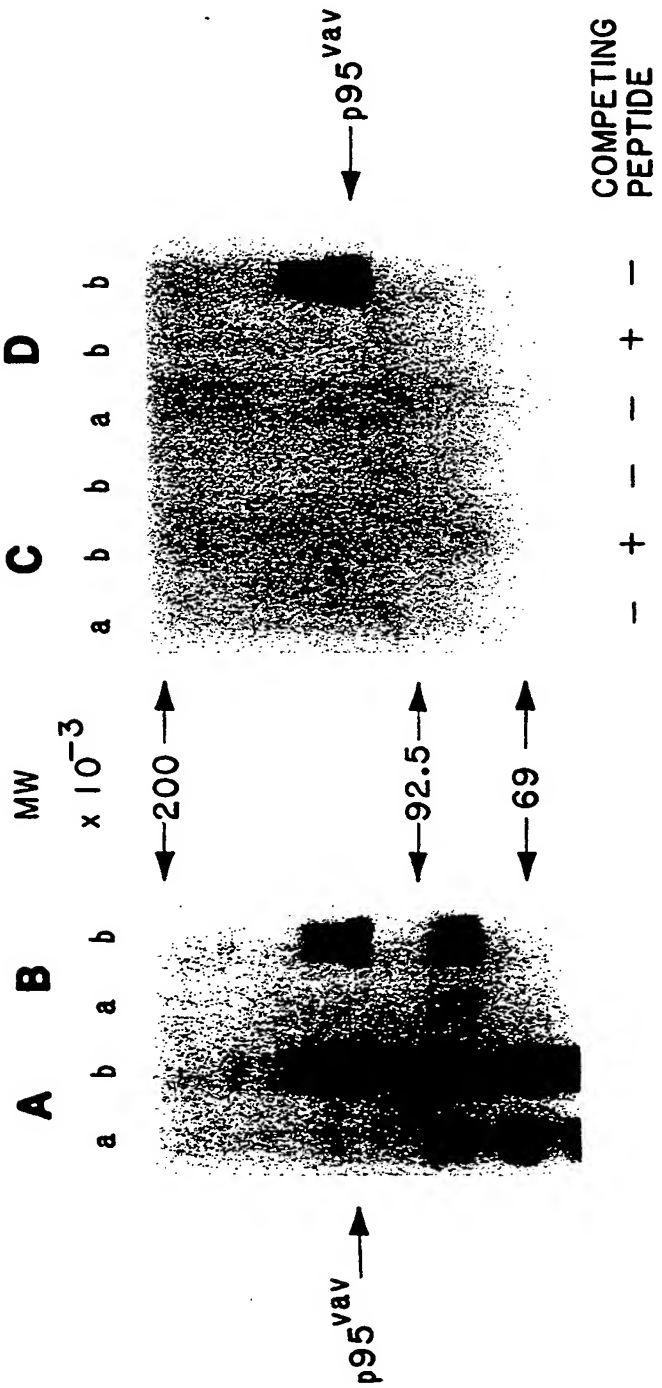
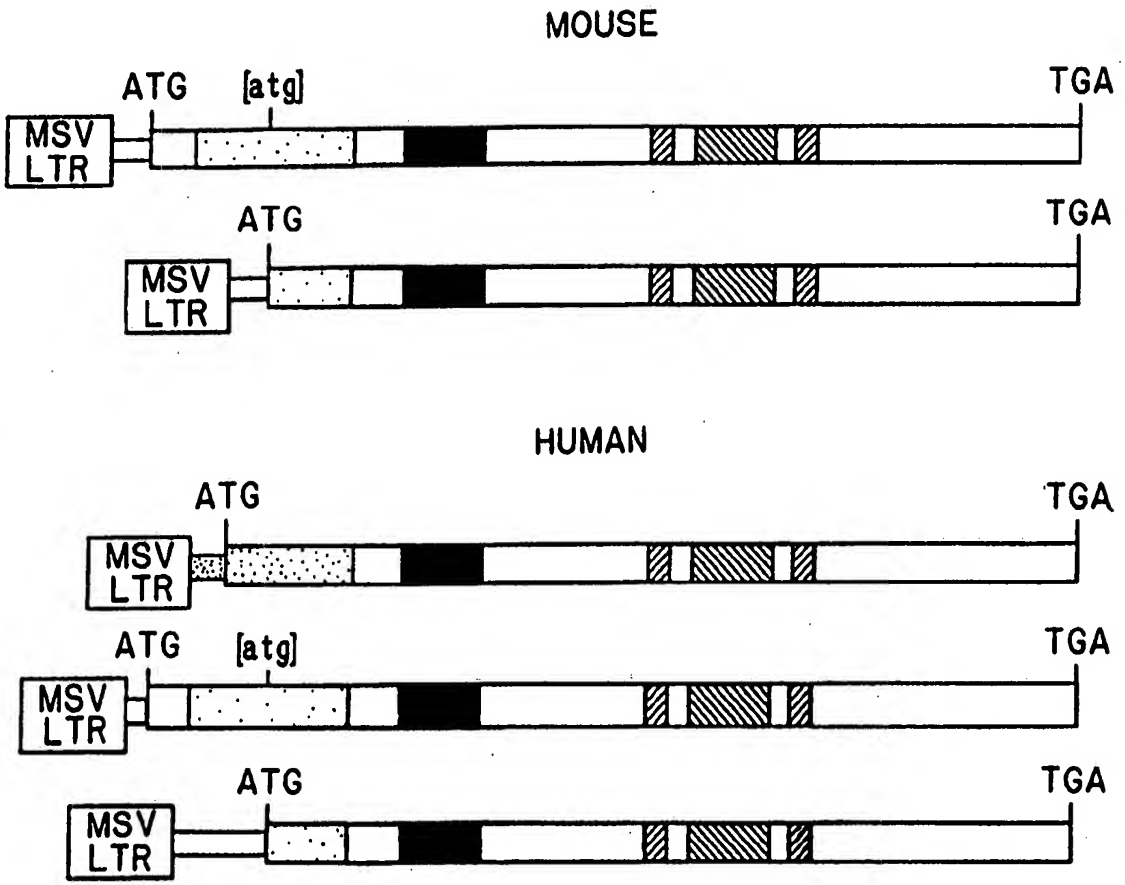


FIG. 4



PLASMID	TRANSFORMING ACTIVITY (ffu/ug DNA)
pJC11	150
pJC12	3,000
pSK27	40,000
pJC13	2,500
pJC7	40,000

FIG. 5

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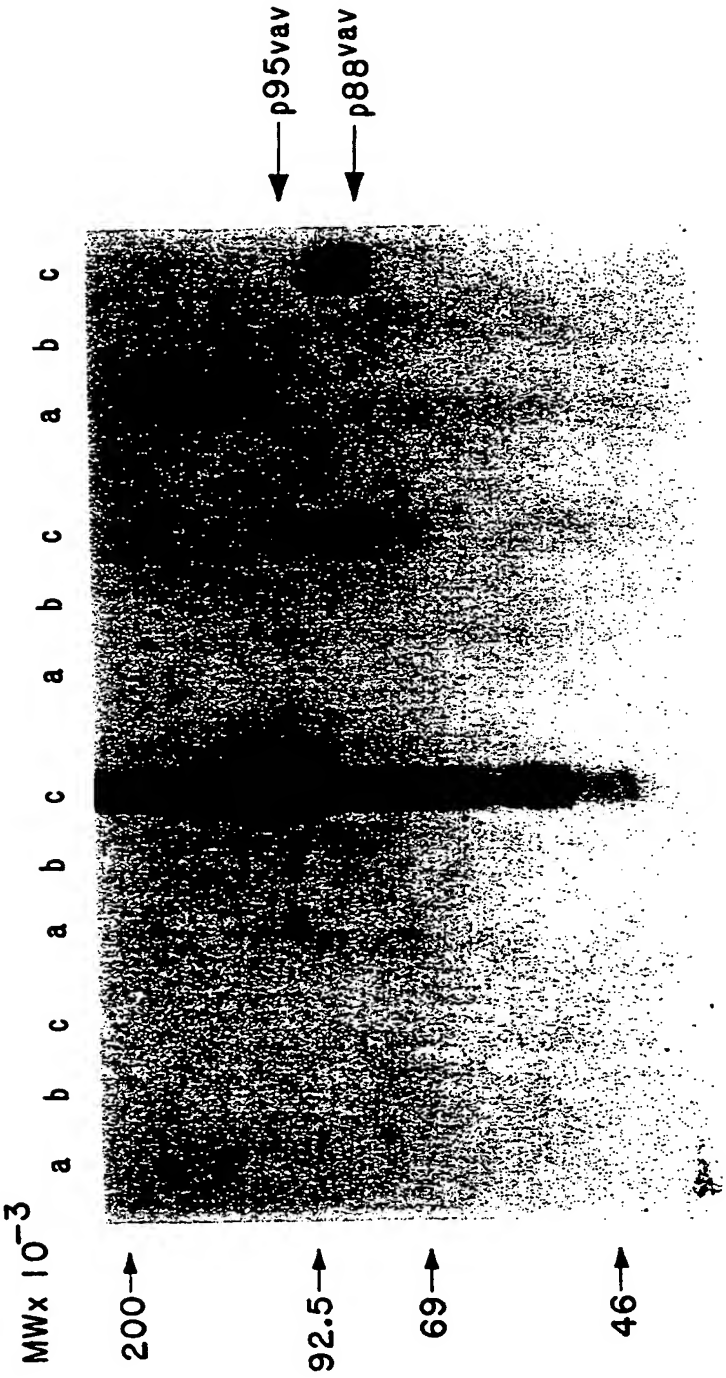

FIG. 6

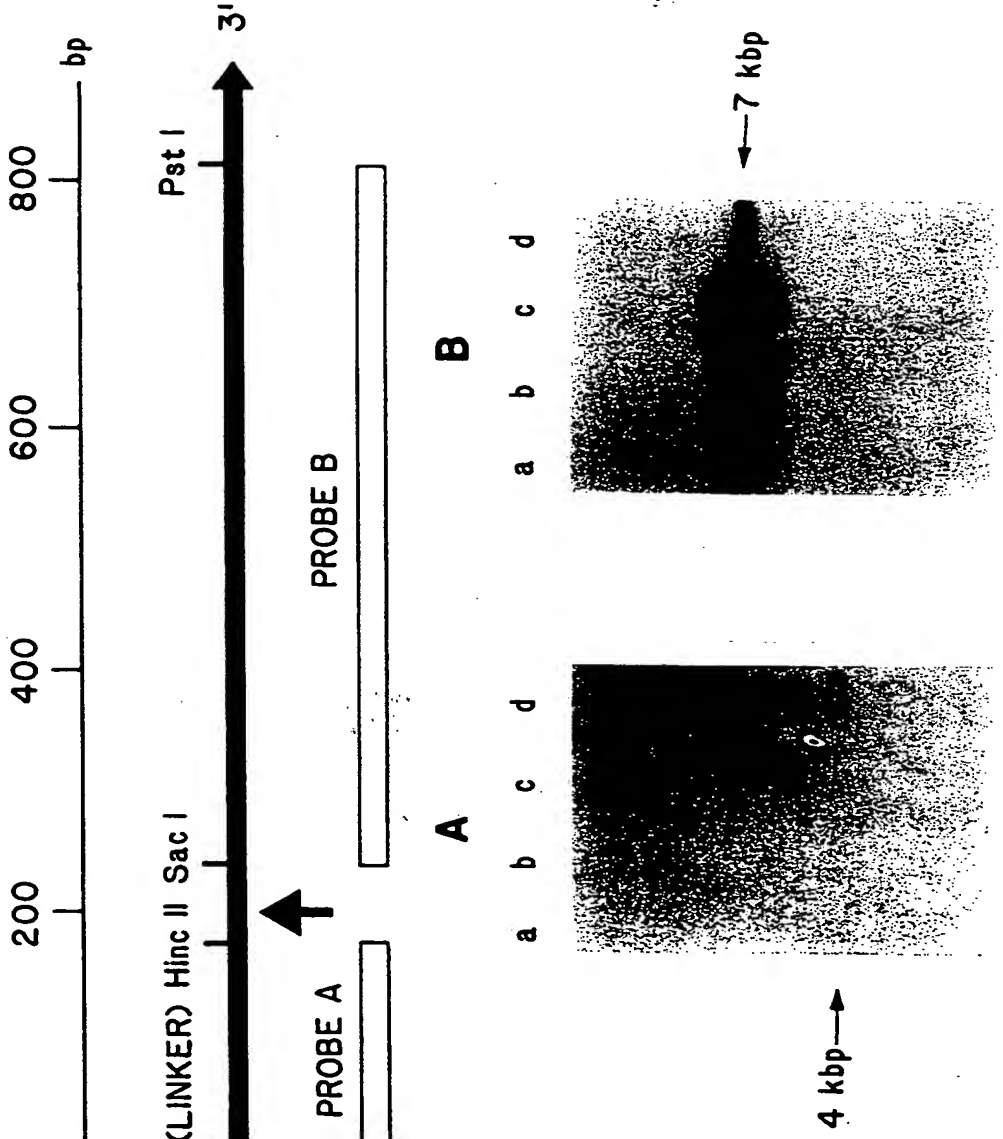

FIG. 7

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 92 30 0557

PAGE1

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	JOURNAL CELLULAR BIOCHEMISTRY SUPPLEMENT VOL. 0 NO. 13 PART B 1989, NEW YORK, US page 80; S. KATZAV ET AL.: 'Activation of a novel human oncogene reveals a locus ubiquitously expressed in cells of hematopoietic lineage' * Abstract * ---	1-26	C12N15/12 C12Q1/68 C12P21/02 C07K15/00
P,X	CELL GROWTH DIFFERENTIATION vol. 2, no. 2, 1991, US pages 95 - 106; J. COPPOLA ET AL.: 'Mechanism of activation of the vav protooncogene' * Whole article * ---	1-26	
P,X	MOLECULAR AND CELLULAR BIOLOGY vol. 11, no. 4, April 1991, WASHINGTON DC, US pages 1912 - 1920; S. KATZAV ET AL.: 'Loss of the amino-terminal helix-loop-helix domain of the vav proto-oncogene activates its transforming potential' * Whole article * ---	1-26	TECHNICAL FIELDS SEARCHED (Int. Cl.5) C07K C12Q
D,Y	EMBO JOURNAL vol. 8, no. 8, 1989, OXFORD, GB pages 2283 - 90; S. KATZAV ET AL.: 'Vav, a novel human oncogene derived from a locus ubiquitously expressed in hematopoietic cells' * Whole article * ---	1-26	
Y	WO-A-8 910 412 (APPLIED BIOTECHNOLOGY, INC. ET AL.) * Whole document * ---	1-26	
A	WO-A-9 014 440 (UNITED STATES OF AMERICA) * Whole document *	1,6, 12-14,23	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	04 MAY 1992	JULIA P.

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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	IMMUNOL. SERIES vol. 41, 1988, NEW YORK, US pages 121 - 148; M. BARBACID: 'Ras oncogenes in human and carcinogen-induced animal tumors' * Whole article, in particular page 122 - 123 and figure 1 *	1,14,23	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)

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